[1]For the polyamine concentrations in each food, the mean concentrations in the following foods were used; [2]Jonagold, Golden, and Granny Smith; [3]Red grape and green grape; [4]Orange and orange (Bardocz); [5]Raisin, prune, pear, peach, apricot, kiwi, strawberry, and melon; [6]French bean, red bean, garden pea, soyabean (Bardocz), and red kidney bean (Bardocz); [7]Hazelnut, almond, and pistachio; [8]Rice, semolina, pasta, white bread, oat bread, rye bread, and whole wheat bread; [9]Potato, skinned; potato with skin; and potato (Bardocz); [10]Maize (Nishibori); [11]Onion and onion (Bardocz); [12]Tomato and tomato (Bardocz); [13]Salsify, celery, carrot, green cabbage, beet, beetroot, carrot, sorrel, radish, chicory, leek, escarole, red cabbage, green leek, Brussels sprout, lettuce, chervil, cabbage, parsley, mushroom, and button mushroom; [14]Garlic, yellow pepper, green pepper, and red pepper; [15]Whisky and Cognac; [16]Lager beer, and stout beer; [17]White (Burgundy), white (Loire), red (Bordeaux), red (Cotes-du-Rhone), red (Touraine), and red (Beaujolais) wines; [18]Veal and beef; [19]Squid and octopus (Nishibori); [20]Soft cheese, Swiss Emmental, French Emmental, goat cheese without rind, Brie pasteurized without rind, graded cheese, Camembert, Brie pasteurized with rind, goat cheese with rind, Roquefort, sweet Cantal with rind, Comte, Saint Nectaire without rind, Saint Nectaire with rind, aged cheddar (Bardocz), and fresh cheddar (Bardocz); [21]Scampi, shrimp, crayfish, and crab claw; [22]Ox tongue, liver mousse, chitterling, duck liver paste, and pork liver paste; [23]Hake, cod, whiting, smoked salmon, mullet, fresh salmon, cod (Bardocz), and trout (Bardocz); [24]Veal, pork, turkey, chicken leg, rabbit, lamb, chicken wing, and beef; [25]Oyster, white scallop, coral scallop, and clam (Nishibori); [26]Hake, cod, whiting, smoked salmon, mullet, fresh salmon, cod (Bardocz), trout (Bardocz), scampi, shrimp, crayfish, crab, squid, octopus (Nishibori), oyster, white scallop, coral scallop, and clam (Nishibori); [27]Turkey wing, chicken leg, and chicken wing; [28]No available data, therefore data of matured yogurt were used. Concentrations of polyamines in foods with no superscript indicate that they were from a single food. Polyamine concentrations were expressed as nmol/g or mL. [23]The amount in fish was a sum of the amounts in freshwater fish, and demersal, pelagic, and other marine fish, and [26]the amount in other marine meat was obtained by subtracting the sum of the amounts in fresh water fish, demersal and pelagic fish, other marine fish, crustaceans, mollusks, and cephalopods from the amount in fish & seafood in the FOSTAT database. Aquatic animals and other aquatic products were not consumed in surveyed countries. Polyamine concentrations in foods were taken from Cipolla B.G., *et al.* Polyamine contents in current foods: a basis for polyamine reduced diet and a study of its long term observance and tolerance in prostate carcinoma patients. Amino Acids 2007; 33: 203-12. Those marked as (Bardocz) were from Bardocz S., *et al.* Polyamines in food-implications for growth and health. J.Nutr.Biochem. 4: 66-71, 1993; and (Nishibori), from Nishibori N., *et al.* Amounts of polyamines in foods in Japan and intake by Japanese. Food Chem 2007; 100: 433-872.

relative amounts of foods as well as the amount of polyamines were compared between higher GDP and lower GDP countries.

2.2. Statistics

Food supply and polyamine amount in higher GDP and lower GDP countries were compared by Mann-Whitney test and p values less than 0.05 were considered significant. Analyses were done using StatView 5.0 (SAS Institute Inc.) run on an Apple computer, and regression coefficients greater than 0.4 and P values of less than 0.05 were considered significant.

3. RESULTS

3.1. Amount and Proportion of Three Food Groups as Sources of Calories, Protein, and Fat

Table 2 shows the amount of calories, protein, and fat of total foods and of three food categories, and **Figure 1** shows the proportions of calories, protein, and fat for three food categories. Higher GDP countries tend to prefer animal products and seafood products more than lower GDP countries. Calories from animal and seafood products represented 29.03 ± 4.55% and 1.56 ± 1.04%, respectively, of total calorie in higher GDP countries and were significantly higher ($p < 0.001$) than those in lower GDP countries (21.61 ± 5.36% and 0.68 ± 0.14%, respectively). Conversely, the proportion of crops calories relative to total calories in lower GDP countries was greater than that in higher GDP countries (77.71 ± 5.66% vs. 69.41 ± 5.13%, $p < 0.001$). Similar to calories, protein from animal, seafood, and crops products accounted for 53.91 ± 4.51%, 7.02 ± 4.15%, and 39.08 ± 5.06%, respectively, in higher GDP countries and 41.67 ± 9.00%, 3.46 ± 3.38% and 54.87 ± 10.68%, respectively, in lower GDP countries (these differences were significant with p values of less than 0.001). The percentages of fat from animals and crops relative to total fat were similar (p = 0.358 and 0.230, respectively) for both higher (53.49 ± 9.92% and 50.31 ± 10.69%) and lower GDP countries (44.55 ± 11.08% and 48.86 ± 10.90%). However, the proportion of fat from seafood relative to total fat was higher ($p < 0.001$) in higher GDP countries (1.96 ± 2.14%) compared to lower GDP countries (0.83 ± 0.74%).

3.2. The Supply of Various Foods per Total Calorie (Table 3)

The majority of the amount of animal and seafood pro-

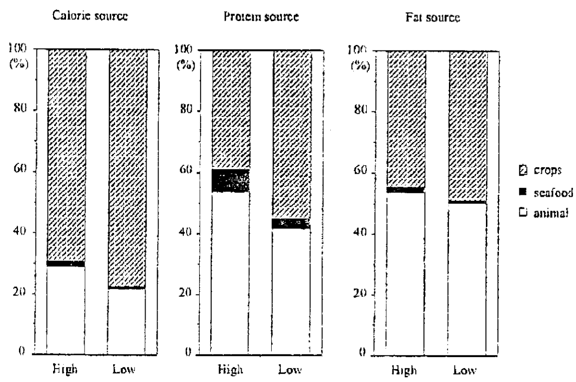

Figure 1. Percentage of calories, protein, and fat from crops, seafood, and animal products relative to total amounts. All data were obtained from the online database of the Statistics Division of the Food and Agriculture Organization of the United Nations (FAOSTAT). "High" indicates higher GDP countries where the GDP (PPPPC) in 2005 was more than 20,000 (current international dollars) and "Low" represents lower GDP countries where the GDP (PPPPC) in 2005 was less than 20,000 (current international dollars).

Table 2. Calorie, protein and fat supply.

	Higher GDP countries	Lower GDP countries	All countries
Calorie supply (kcal/capita/day)			
Animal calorie	996.22 ± 137.00	655.07 ± 210.23	829.13 ± 245.47
Seafood calorie	53.10 ± 33.88	21.88 ± 23.67	37.81 ± 33.03
Crops calorie	2397.57 ± 276.63	2310.54 ± 255.59	2354.94 ± 267.39
Total calorie	3446.89 ± 204.59	2987.49 ± 378.23	3221.88 ± 378.57
Protein supply (g/capita/day)			
Animal protein	57.93 ± 5.98	36.96 ± 11.45	47.66 ± 13.89
Seafood protein	7.68 ± 5.20	3.31 ± 3.78	5.54 ± 7.84
Crops protein	42.04 ± 6.65	47.16 ± 8.26	44.55 ± 7.84
Total protein	107.65 ± 9.13	87.42 ± 14.47	97.74 ± 15.70
Fat supply (g/capita/day)			
Animal fat	75.05 ± 12.63	47.36 ± 17.04	61.49 ± 20.36
Seafood fat	2.73 ± 2.93	0.84 ± 0.83	1.80 ± 2.36
Crops fat	63.77 ± 19.49	45.34 ± 13.96	54.74 ± 19.24
Total fat	141.55 ± 14.59	93.53 ± 25.17	118.03 ± 31.60

Data are expressed mean ± standard deviation (SD). Higher GDP countries indicate countries where GDP was greater than 20,000 (current international dollars). Lower GDP countries indicate countries where GDP values less than 20,000 (current international dollars).

ducts per total calorie was higher in higher GDP countries than in lower GDP countries. While supply of dairy products, especially cheese, was greater in higher GDP countries than lower GDP countries, whole milk supply per calorie was significantly higher in lower GDP countries than higher GDP countries. The majority of crops supply per calorie was higher in lower GDP countries, although fruit and tomato supply was greater in higher GDP countries compared to lower GDP countries. In addition, alcoholic drinks, especially wine and beer, were preferred in greater amounts in higher GDP countries relative to lower GDP countries.

3.3. Amount and Proportion of Three Polyamines

The average amounts of spermine, spermidine, and putrescine in foods were 38.48 (range 17.61-54.82), 89.05 (range 59.69-132.23), and 184.32 (range 71.82-419.17) µmol/day/capita, respectively, in all targeted countries; 46.23 ± 5.37, 90.87 ± 15.72, and 236.58 ± 69.47 µmol/day/capita, respectively, in higher GDP countries and 30.39 ± 8.09, 87.15 ± 16.28, and 129.89 ± 37.59 µmol/day/capita, respectively, in lower GDP countries.

When the proportions of each of the three polyamines accounting for total polyamines were compared (**Figure 2**), the percentage of spermine was similar (p = 0.810) between the two groups of countries (12.84 ± 2.68% in higher GDP countries, and 12.41 ± 2.45% in lower GDP countries), and the proportion of spermidine was significantly lower in higher GDP countries compared to lower GDP countries (24.75 ± 3.35% vs. 35.64 ± 4.24%, p < 0.001), while putrescine was significantly higher in higher GDP countries relative to lower GDP countries (62.41 ± 5.52% vs. 51.96 ± 4.37%, p < 0.001).

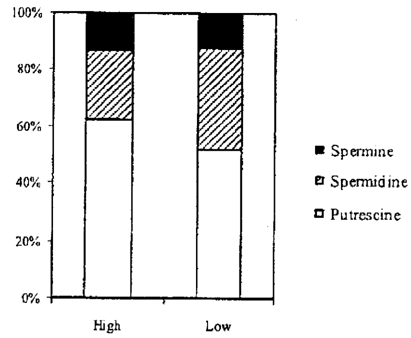

Figure 2. Percentage of spermine, spermidine, and putrescine relative to total polyamine intake in higher GDP countries (High, countries where GDP in 2005 was more than 20,000) and lower GDP countries (Low, countries where GDP in 2005 was less than 20,000). The polyamine amounts were calculated using values from public databases. Dietary data were gathered from FAOSTAT, and polyamine concentrations are indicated in **Table 1**.

Table 3. Calorie, protein and fat supply.

	Higher GDP countries	Lower GDP countries	*p*-value
	A. Animal meat (g) per total calorie (1000*kcal*)		
Bovine	17.09 ± 6.95	11.15 ± 5.40	0.004
Pork	28.56 ± 11.48	15.40 ± 12.20	< 0.001
Mutton&Goat	4.13 ± 5.76	2.75 ± 3.99	0.39
Poultry	21.08 ± 10.46	11.98 ± 6.10	< 0.001
Offals	3.05 ± 2.20	3.26 ± 1.52	0.459
Other meats	1.73 ± 1.70	0.66 ± 1.03	0.001
Dairy products	199.17 ± 49.43	164.73 ± 45.12	0.012
Cheese	12.49 ± 4.94	4.58 ± 3.08	< 0.001
Whole milk	65.98 ± 30.02	110.40 ± 52.25	< 0.001
Butter & Ghee	2.76 ± 1.62	1.61 ± 1.19	0.009
Honey	0.59 ± 0.30	0.52 ± 0.39	0.327
Egg	8.70 ± 2.49	8.66 ± 3.09	0.81
	B. Seafoods (g) per total calorie (1000*kcal*)		
Demersal fish	6.57 ± 5.03	2.50 ± 5.03	< 0.001
Pelagic fish	6.38 ± 7.47	3.92 ± 4.31	0.02
Fresh water fish	2.86 ± 2.17	1.13 ± 0.87	< 0.001
Other marine fish	0.79 ± 0.76	0.60 ± 0.73	0.418
All fish	16.60 ± 10.69	8.15 ± 8.23	< 0.001
Molluscs	1.61 ± 1.56	0.24 ± 0.53	< 0.001
Cephalopods	0.82 ± 1.30	0.25 ± 0.66	0.017
Crustaceans	2.64 ± 3.00	0.55 ± 1.47	< 0.001
Seafood total	21.73 ± 13.61	9.22 ± 9.66	< 0.001
	C. Crops (g) per total calorie (1000*kcal*)		
Cereals	93.06 ± 16.67	147.14 ± 42.34	< 0.001
Fruits	97.27 ± 22.47	61.25 ± 23.73	< 0.001
Vegetables	94.21 ± 36.04	127.12 ± 51.14	0.083
(Fruits&Vegetables)	191.47 ± 49.29	188.37 ± 65.83	0.447
Pulses	2.42 ± 1.60	2.04 ± 2.47	0.139
Potato (All)	55.57 ± 14.46	75.16 ± 34.67	0.052
Tomato	16.87 ± 9.85	8.28 ± 9.61	< 0.001
Beer	61.03 ± 29.36	38.31 ± 25.40	0.008
Wine	16.87 ± 9.85	8.28 ± 9.61	< 0.001

Data are expressed mean ± standard deviation (SD). Higher GDP countries indicate countries where GDP was greater than 20,000 (current international dollars). Lower GDP countries indicate countries where GDP values less than 20,000 (current international dollars).

Foods in higher GDP countries seemed to contain spermine and putrescine in greater amounts than those in lower GDP countries (**Table 4**). Conversely, foods in lower GDP countries tended to contain spermidine in much greater amounts compared to higher GDP countries. Simple regression analyses revealed that GDP has positive correlations with total polyamine per total calorie ($r = 0.503$, $p < 0.01$), total spermine per total calorie ($r = 0.677$, $p < 0.01$), and total putrescine per total calorie ($r = 0.608$, $p < 0.01$). However, there was a negative

Table 4. Comparison of polyamine amount (μmol) per total calorie (1000 kcal/day).

	Higher GDP countries	Lower GDP countries	p-value
Spermine	13.43 ± 1.54	10.07 ± 1.76	< 0.001
Spermidine	26.30 ± 3.77	29.35 ± 5.45	0.018
Putrescine	68.43 ± 18.38	43.26 ± 9.91	< 0.001
SPM + SPD	39.73 ± 4.25	39.42 ± 5.39	0.719
Total polyamine	108.16 ± 21.11	82.69 ± 13.63	< 0.001

Data are expressed mean ± standard deviation (SD). Higher GDP countries indicate countries where GDP was greater than 20,000 (current international dollars). Lower GDP countries indicate countries where GDP values less than 20,000 (current international dollars). SPM: spermine; SPD: spermidine.

correlation between GDP and total spermidine per total calorie (r = –0.498, $p < 0.01$). Individuals in higher GDP countries preferred foods rich in polyamine, especially spermine and putrescine, while individuals in lower GDP countries preferred foods rich in spermidine.

3.4. Proportion of Three Food Groups as Sources of Three Polyamines (Figure 3)

The high percentage (73.26 ± 4.57%) of food-based spermine originated in animal products in higher GDP countries, and its proportion was significantly higher ($p < 0.001$) than that for lower GDP countries (55.40 ± 13.72%). Spermine from crops represented 23.05 ± 4.20% and 43.24 ± 14.35% of total spermine in higher and lower GDP countries, respectively ($p < 0.001$). The majority of spermidine and putrescine originated in crops; 83.85 ± 3.61% of spermidine and 83.74 ± 5.57% of putrescine in higher GDP countries, and 92.59 ± 3.49% of spermidine and 89.86 ± 5.39% of putrescine in lower GDP countries. The proportion of crops spermidine and putrescine relative to total amounts was higher ($p < 0.001$ for both) in lower GDP countries compared to higher GDP countries. Spermidine from animal products accounted for 14.57 ± 3.28% and 6.94 ± 3.16% of total spermidine, while the percentage of putrescine from animal products was 14.10 ± 5.53% and 9.12 ± 5.01% of total putrescine in high and lower GDP countries, respectively ($p < 0.001$ for spermidine and $p = 0.003$ for putrescine). The amounts of spermine, sper midine, and putrescine from seafood in each total amount were small: 3.70 ± 2.03%, 1.59 ± 0.94%, and 2.16 ± 1.12%, respectively, for higher GDP countries, and were only 1.36 ± 1.99%, 0.47 ± 0.75%, and 1.02 ± 1.26%, respectively, for lower GDP countries. The percentages for higher GDP countries were greater than those for lower GDP countries ($p < 0.001$). Individuals in higher GDP countries took these three polyamines from animal and seafood products much more than did

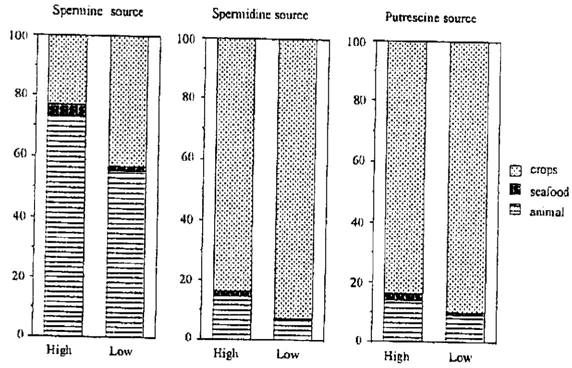

Figure 3. Percentage of crops, seafood, and animal products relative to total amounts of spermine, spermidine, and putrescine in higher GDP countries (High) and lower GDP countries (Low).

those in lower GDP countries, while lower GDP countries obtained polyamines from crops.

4. DISCUSSION

Differences in socioeconomic status are known to affect the dietary pattern of individuals [22-25]. In this ecological study, we illustrate the relationship between GDP and dietary pattern on the basis of country. The results of the study where data were obtained from open databases have several similarities to those of previous epidemiological studies using personal and collective databases. Namely, higher socioeconomic status is associated with increased intake of fruits, seafood, and cheese.

In the present study, despite the higher supply of crops products in lower GDP countries compared to higher GDP countries, fruits are preferred in higher GDP countries. It is widely accepted that higher socioeconomic status is associated with increased intake of fruits and vegetables [11,23,26-31]. Similarly, as observed in the present study, many studies have shown a positive association between socioeconomic status and seafood intake [28-29,32]. Although the association between dairy products and socioeconomic status is not so apparent, some epidemiological studies have shown that skimmed milk is mainly consumed by the higher socioeconomic groups whereas the lower groups consume full-fat milk [11,31]. While we had insufficient information about the fat content of dairy products, our findings that individuals in higher GDP countries consumed more cheese than whole milk are consistent with previous studies [22,30].

This study delineates the relationship between food polyamines and socioeconomic status of countries. The absolute amounts of three polyamines in all targeted countries obtained from database information are also similar to those of the previous studies in which about 35 μmol spermine, 55 μmol spermidine, and 160 μmol putrescine were estimated to be consumed [33], and those in higher GDP countries were also similar to those of previous reports for higher GDP countries, Britain, Italy, Spain, Sweden, and Netherlands in which 350 to 500 μmol polyamines were estimated to be consumed [34].

The present study shows that individuals in higher GDP countries prefer foods rich in polyamine, especially spermine and putrescine, much more than those in lower GDP countries. Increased spermine supply in higher GDP countries seems due mainly to the increased supply of animal meat, in which spermine is abundant. Increased putrescine supply in higher GDP countries seems to be due to the increased supply of vegetables and fruit, where the putrescine concentration is high.

This ecological study showed that socioeconomic status is associated not only with dietary pattern but also with the amount and proportion of polyamines. The difference in food choice is considered to have some role in the prevalence of several diseases [35-47], and our previous studies showed that increased polyamine intake contributes to decreases in age-associated pathological changes in mice [13]. Therefore, increased polyamine intake may have some role on the difference in the prevalence of diseases associated with socioeconomic disparity. However, this is an ecological study and data do not necessarily indicate the personal food consumption, so, there may be confounding factor(s) between polyamine amount and socioeconomic status. Further analyses using personal database are desired.

5. ACKNOWLEDGEMENTS

Statement of conflicts of interest and funding: We have no conflict of interest to disclose. Sources of funding: This research received no specific grant from any funding agency in the public, commercial, or non-profit sectors.

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Nutrition and Dietary Supplements

Dove

ORIGINAL RESEARCH

Mediterranean diet and polyamine intake: possible contribution of increased polyamine intake to inhibition of age-associated disease

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Abstract: The Mediterranean diet is a dietary pattern associated with increased longevity, and has been shown to have anti-inflammatory properties. Based on the findings that natural polyamines are strong anti-inflammatory substances, we have found that continuous and increased polyamine intake prolongs murine lifespan. Because polyamines are contained in most foods in widely varying concentrations, we sought epidemiologic evidence that supports an association between the Mediterranean diet and increased polyamine intake. The amounts of food supply in 49 European and other Western countries in 2005 were collected from the United Nations database, and the amount of food polyamine was estimated using polyamine concentrations in foods from published sources. The Mediterranean diet pattern was characteristically observed in Mediterranean countries. For all 49 countries and for foods such as olive oil (Spearman $r = 0.602$), fruit ($r = 0.804$), fruit and vegetables ($r = 0.611$), seafood ($r = 0.461$), and cheese ($r = 0.411$), the ratios of the amounts of these foods to total calories consumed were all positively associated ($P < 0.05$) with the amount of polyamine per calorie. Legumes per calorie ($r = 0.379$), wine per calorie ($r = 0.285$), and the amount of seafood and poultry meat relative to red meat ($r = 0.313$) had a trend of positive association with the amount of polyamine per calorie ($P < 0.05$), while several foods in the non-Mediterranean diet group had a trend of no or negative association. Food polyamines are absorbed quickly from the intestinal lumen, and long-term increased polyamine intake increases blood polyamine concentration. The present findings, together with previous studies on polyamines, indicate a possible role for the food polyamines that are abundant in the Mediterranean diet in prolonging human life.

Keywords: Mediterranean diet, polyamine, longevity, age-associated diseases

Introduction

The phrase "Mediterranean diet" reflects the dietary patterns characteristic of several countries in the Mediterranean Basin. In spite of the relatively high amount of fat consumed in Mediterranean countries, individuals living in these countries have far lower rates of cardiovascular disease than do those living in the surrounding European countries and the US, where similar levels of fat are consumed. A large number of epidemiologic studies, as well as several interventional studies, have shown that the Mediterranean diet pattern is closely associated with prolonged lifespan and decreased mortality due to chronic age-associated health deterioration.[1–6]

The principal components of the Mediterranean diet pattern include olive oil (the principal source of fat), high consumption of legumes, fruit, and vegetables, with moderate consumption of dairy products (mostly as cheese and yoghurt), moderate to high consumption of fish and poultry, low consumption of red meat and meat

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products, as well as low to moderate wine consumption. Several nutrients and non-nutrients have been shown to be abundant in the Mediterranean diet pattern, and their possible contributions to decreased mortality due to age-associated diseases have been investigated. However, despite these extensive studies, the role of nutrients such as antioxidants, isoflavone, vitamins, and phytochemicals in preventing disease has not been successfully confirmed. Consequently, the focus has turned to whole diets rather than specific supplements to prevent age-associated diseases, such as cardiovascular disease.[7]

Polyamines (spermine, spermidine, and putrescine) are organic compounds having two or more primary amino groups, are indispensable for cell growth and differentiation, and are contained in almost all cells. Polyamine is absorbed quickly from the intestinal lumen and distributed to all organs and tissues in the body.[8–11] Because most foods originate from plants or animals, almost all foods contain polyamines, but in widely varying concentrations.[12–14] Among various foods, beans, especially soybeans, contain abundant polyamines, especially spermine and spermidine. A large number of studies have shown that soybeans contain component(s) that have favorable effects on the progression of chronic age-associated disease, although the identity of these substances remains undefined.[15]

We have shown that spermine and spermidine exert an anti-inflammatory effect by inhibiting the synthesis of proinflammatory cytokines and decreasing exclusively the expression of leukocyte function-associated antigen-1, one of the pivotal molecules needed to elicit immune cell activation and inflammation.[16,17] Considering the findings that anti-inflammatory substances, such as component(s) of the Mediterranean diet, soybeans, and n-3 unsaturated fatty acid found in fish oil seem to inhibit age-associated diseases, then anti-inflammatory polyamines may have a role in inhibiting the progression of age-associated disease.[18–22] This is supported by our finding that mice having a long-term intake of chow with polyamine concentrations two to three times higher than that of soybeans had decreased age-associated pathologic changes and increased longevity.[23,24]

Materials and methods

In order to support the premise that the increased polyamine intake by the Mediterranean diet helps inhibit age-associated diseases and increase human longevity, epidemiologic evidence indicating an association between Mediterranean diet and increased polyamine intake was gathered. Dietary data (levels of food supply in 2005) were obtained from the online database of the Statistics Division of the Food and Agriculture Organization of the United Nations. The target populations were those of 49 countries in Europe, North America, and Oceania (see Figure 1) with similar racial and ethnic composition, as well as social and religious backgrounds.

The food concentrations of spermine, spermidine, and putrescine were obtained from published reports of concentrations measured in European foods.[12,13] When these reports lacked polyamine concentrations for specific foods, or additional data were necessary to obtain an accurate average concentration in a food, we used data from Nishibori et al.[14]

Because food supply data from the World Health Organization do not necessarily indicate the absolute amount consumed by each nation and in order to capture the features of the dietary pattern, a relative measure of the amount of various foods, such as food supply per total calories, was employed.

The availability of foods in Mediterranean countries and northern European countries was compared by Mann-Whitney U test, with a P value less than 0.05 considered significant. Spearman correlation coefficients were calculated to examine the association between the relative amount of various foods and polyamine amount per calorie. Analyses were done using StatView 5.0 (SAS Institute Inc, Cary, NC) run on an Apple computer, with correlation coefficients of more than 0.4 and P values less than 0.05 considered to be significant.

Results

The concentrations of three polyamines obtained from published papers and used for the present study are

Albania, Armenia, Australia, Austria, Azerbaijan, Belarus, Belgium, Bosnia and Herzegovina, Bulgaria, Canada, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Georgia, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Kazakhstan, Latvia, Lithuania, Malta, The Netherlands, New Zealand, Norway, Poland, Portugal, Romania, Russian Federation, Slovakia, Slovenia, Spain, Sweden, Switzerland, Tajikistan, The former Yugoslav Republic of Macedonia, Turkey, Turkmenistan, Ukraine, United Kingdom, United States of America, Uzbekistan

Figure 1 List of target countries.

Table 1 Concentrations of three polyamines in foods[a]

	Spermine	Spermidine	Putrescine
Apple[b]	0	14.73	14.27
Banana	1.00	44.90	317.30
Lemon and lime	0.90	18.40	53.80
Citrus (other)	0.90	18.40	53.80
Pineapple	10.90	27.00	7.60
Grape[c]	1.60	22.50	26.25
Orange and mandarin[d]	0	41.40	1143.35
Other fruit[e]	3.02	25.50	11.55
Pulses[f]	66.46	179.70	69.64
Treenuts[g]	46.93	186.97	56.90
Groundnut	34.60	388.70	61.40
Cereals[h]	17.94	57.55	27.29
Potato[i]	7.90	64.70	68.73
Maize[j]	8.00	144.00	576.00
Onion[k]	2.50	41.20	38.85
Tomato[l]	0	19.35	380.20
Vegetables[m]	6.69	124.13	52.98
Stimulants[n]	12.50	61.40	18.98
Oil crops	0	0	0
Sugar	0	0	3.00
Coffee	0	0	0
Alcoholic beverages[o]	0	1.00	0
Beer[p]	0	0.50	18.60
Wine[q]	0	2.17	26.80
Animal fats	0	0	0
Beef[r]	120.70	22.45	36.00
Butter and ghee	0	0.50	0
Cephalopods[s]	86.00	13.50	82.00
Cheese[t]	21.58	145.34	589.71
Cream	0	0	0.30
Crustaceans[u]	0	1.98	4.48
Edible offals[v]	98.90	82.28	11.34
Eggs	0	0	20.50
Fish[w]	16.25	16.35	61.93
Honey	0	1.00	8.00
Meat[x]	110.53	29.68	32.78
Molluscs[y]	94.43	73.13	202.83
Mutton and goat meat	131.30	39.70	8.20
Other marine meat[z]	37.76	25.46	82.70
Pork	160.15	18.15	19.50
Poultry[za]	91.70	27.50	11.43
Whey[zb]	1.00	1.00	0
Whole milk	0	0	0.30

Notes: [a]For the polyamine concentrations in each food, the mean concentrations in the following foods were used: [b]Jonagold, Golden, and Granny Smith; [c]red grape and green grape; [d]orange and orange[9]; [e]raisin, prune, pear, peach, yellow peach, dates, kiwifruit, strawberry, and melon; [f]French bean, red bean, garden pea, soyabean[9], and red kidney bean[9]; [g]hazelnut, almond, and pistachio; [h]rice, semolina, pasta, white bread, oat bread, rye bread, and whole wheat bread; [i]potato skinned, potato with skin, and potato[9]; [j]maize[14]; [k]onion and onion[9]; [l]tomato and tomato[9]; [m]salsify, celery, carrot, green cabbage, beet, beetroot, carrot, sorrel, radish, chicory, leek, escarole, red cabbage, green leek, Brussels sprout, lettuce, chervil, cabbage, parsley, mushroom, and button mushroom; [n]garlic, yellow pepper, green pepper, and red pepper; [o]whisky and cognac; [p]lager beer and stout beer; [q]white (Burgundy), white (Loire), red (Bordeaux), red (Côtes du Rhône), red (Touraine), and red (Beaujolais) wines; [r]veal and beef; [s]squid and octopus[14]; [t]soft cheese, Swiss Emmental, French Emmental, goat cheese without rind, Brie pasteurized without rind, graded cheese, Camembert, Brie pasteurized with rind, goat cheese with rind, Roquefort, sweet Cantal with rind, Comte, Saint Nectaire without rind, Saint Nectaire with rind, aged Cheddar[9], and fresh cheddar[9]; [u]scampi, shrimp, crayfish, and crab claw; [v]ox tongue, liver mousse, chitterling, duck liver paste, and pork liver paste; [w]hake, cod, whiting, smoked salmon, mullet, fresh salmon, cod[9], and trout[9]; [x]veal, pork, turkey, chicken leg, rabbit, lamb, chicken wing, and beef; [y]oyster, white scallop, coral scallop, and clam[14]; [z]hake, cod, whiting, smoked salmon, mullet, fresh salmon, cod[9], trout[9], scampi, shrimp, crayfish, crab, squid, octopus[14], oyster, white scallop, coral scallop, and clam[14]; [za]turkey wing, chicken leg, and chicken wing. [zb]No available data, therefore data of matured yoghurt were used. Concentrations of polyamines in foods with no superscript indicate that they were from a single food[13]. Polyamine concentrations were expressed as nmol/g or mL. The amount in fish was a sum of the amounts in freshwater fish, and demersal, pelagic, and other marine fish; the amount in other marine meat was obtained by subtracting the sum of the amounts in fresh water fish, demersal and pelagic fish, other marine fish, crustaceans, molluscs, and cephalopods from the amount in fish and seafood in the FOSTAT database. Aquatic animals and other aquatic products were not consumed in surveyed countries.

shown in Table 1. These concentrations differed considerably among foods. Basically, meat and some seafood contained relatively large amounts of spermine and spermidine, and several fruit and vegetables contained relatively large amounts of spermidine and putrescine. While whole milk had few polyamines, cheese contained a large amount.

To test whether the data showed that increased amounts of Mediterranean diet foods were preferred in Mediterranean countries, food amounts relative to calorie amounts were compared between Mediterranean and northern Europe. As shown in Table 2, greater amounts of olive oil, legumes, vegetables, and fruit and vegetables are preferred in Mediterranean countries compared with northern European countries. In addition, although no significance was observed, Mediterranean countries tend to prefer cheese rather than whole milk, and wheat (cereals) rather than potatoes. While beer and other alcoholic beverages were preferred in northern European countries, wine was preferred in Mediterranean countries. Compared with those in Mediterranean countries, people in northern European countries had a significant preference for foods that are absent from the Mediterranean diet, such as animal fat and potatoes.

The association between the relative amount of Mediterranean diet foods and polyamines per calorie was evaluated for the 49 Western countries. As shown in Table 3, polyamine amount per calorie was significantly associated with the amount of olive oil, fruit and vegetables, seafood, and cheese relative to total calories supplied. Although their correlation coefficients were less than 0.4, the following ratios (legumes to total calories consumed, seafood and poultry to red meat, seafood fat to total fat, wine to total calories consumed) had a trend of positive association with polyamine amount per calorie with P values less than 0.05.

Table 2 Comparison of foods between Mediterranean countries and northern European countries[a]

	Mediterranean[b] countries	Northern[c] countries	P value
Olive oil/total calories	8.34 ± 4.81	0.47 ± 0.37	0.003
Percent olive oil/total fat	19.58 ± 11.76	1.19 ± 0.92	0.005
Percent animal fat/total fat	44.00 ± 8.70	58.76 ± 4.79	0.017
Legumes/total calories	3.16 ± 1.13	1.36 ± 0.78	0.017
Fruit/total calories	105.36 ± 26.89	85.83 ± 23.48	0.257
Vegetables/total calories	140.94 ± 36.70	73.77 ± 13.44	0.003
Fruit and vegetables/total calories	246.30 ± 31.31	159.60 ± 20.20	0.007
Wheat/total calories	91.24 ± 18.42	75.00 ± 17.14	0.089
Potato/total calories	48.00 ± 12.59	77.67 ± 21.51	0.017
Cheese/total calories	14.70 ± 6.75	11.61 ± 5.93	0.497
Dairy products/total calories	182.67 ± 33.94	227.60 ± 49.71	0.141
Whole milk/total calories	54.76 ± 23.62	87.87 ± 32.50	0.070
Percent cheese/dairy products	7.67 ± 2.77	5.09 ± 2.53	0.113
Seafood/total calorie	23.72 ± 8.34	24.76 ± 17.54	0.651
Percent seafood and poultry/red meat	67.62 ± 13.19	83.70 ± 30.59	0.308
Wine/total calories	30.93 ± 9.42	11.60 ± 5.88	0.006
Beer/total calories	32.88 ± 20.13	69.47 ± 5.72	0.017
Other alcoholic drinks/total calories	1.91 ± 1.05	5.17 ± 3.13	0.017

Notes: [a]Data represent mean ± standard deviation; [b]France, Spain, Italy, and Greece; [c]Denmark, Estonia, Finland, Germany, Iceland, Ireland, Latvia, Lithuania, Netherlands, Norway, Russian Federation, Sweden, and UK. Legumes, fruits, vegetables, wheat, potato, cheese, dairy products, and seafood amounts are expressed in µg. Olive oil, whole milk, wine, beer, and other alcoholic drink amounts are expressed in µL.

Table 3 Correlation between polyamine and foods

	Spearman's correlation coefficient	P value
Mediterranean diet foods		
Olive oil/total calories	0.602	<0.001
Olive oil/total fat	0.612	<0.001
Legumes/total calories	0.379	0.009
Legumes/crops calories	0.395	0.006
Fruit/total calories	0.804	<0.001
Fruit and vegetables/total calories	0.611	<0.001
Wheat/total calories	−0.287	0.047
Seafood calories/total calories	0.461	0.001
Seafood and poultry meat/red meat	0.313	0.030
Cheese/total calories	0.411	0.005
Seafood fat/total fat	0.391	0.007
Wine/total calories	0.285	0.049
Non-Mediterranean diet foods		
Potato/total calories	−0.078	0.586
Animal fat/total fat	−0.004	0.980
Whole milk/total calories	−0.323	0.025
Whole milk/dairy products	−0.351	0.015
Beer/total calories	−0.013	0.927
Other alcoholic drink/total calories	−0.136	0.345

Notes: Spearman's correlation coefficients were calculated to examine the association between the relative amount of various foods and polyamine intake per calories. Correlation coefficients of more than 0.4 and a P value less than 0.05 were considered significant. Mediterranean diet foods indicate the typical foods of which consumption were reported to be higher in Mediterranean countries than non-Mediterranean countries.

Among the Mediterranean diet foods, only the ratio of wheat to total calories had a trend of negative association with polyamine amount. In spite of the positive association between cheese and polyamine amount, the ratio of whole milk to total calories and whole milk to dairy products had a trend of negative association with polyamine amount (*P* value less than 0.05).

Because our previous experiments showed that spermine has the most potent biologic activity among natural polyamines,[17] this analysis was done using spermine amount per calorie instead of total polyamine per calorie. As shown in Table 4, the results were similar to those in Table 3. Namely, spermine amount per calorie was significantly associated with supply of many Mediterranean diet foods, such as olive oil, fruit, seafood, cheese, and wine, relative to total calories. Again, despite the positive association between cheese and spermine amount, the ratio of whole milk to total calories and whole milk to dairy products had a negative association with spermine amount.

Table 4 Correlation between spermine and foods

	Spearman's correlation coefficient	*P* value
Mediterranean diet foods		
Olive oil/total calories	0.608	<0.001
Olive oil/total fat	0.579	<0.001
Legumes/total calories	0.270	0.153
Legumes/crops calories	0.226	0.117
Fruit/total calories	0.491	<0.001
Fruit and vegetables/total calories	0.107	0.459
Wheat/total calories	–0.579	<0.001
Seafood calories/total calories	0.587	<0.001
Seafood and poultry meat/red meat	0.140	0.334
Cheese/total calories	0.550	<0.001
Seafood fat/total fat	0.490	<0.001
Wine/total calories	0.511	<0.001
Non-Mediterranean diet foods		
Potato/total calories	–0.100	0.487
Animal fat/total fat	0.160	0.267
Whole milk/total calories	–0.482	<0.001
Whole milk/dairy products	–0.557	<0.001
Beer/total calories	0.464	0.001
Other alcoholic drink/total calories	0.003	0.984

Notes: Spearman's correlation coefficients were calculated to examine the association between the relative amount of various foods and spermine intake per calories. Correlation coefficients of more than 0.4 and a *P* value less than 0.05 were considered statistically significant. Mediterranean diet foods indicate typical foods of which consumption were reported to be higher in Mediterranean countries than in non-Mediterranean countries.

Discussion

The present epidemiologic study using data collected from an open database produced results similar to those of previous reports.[4–6] Namely, in Mediterranean countries, olive oil, legumes, fruit and vegetables, cheese, and wine are consumed in preference to animal fat, potatoes, and whole milk. Unfortunately, some of the data relevant to the Mediterranean diet, such as the amount of unrefined cereals and yoghurt, especially low-fat yoghurt, could not be obtained. Also, there was no between-region difference in the supply of a few Mediterranean diet foods per total calories, such as seafood and poultry relative to red meat. However, this epidemiologic study using an open database revealed that most Mediterranean diet foods are preferred in Mediterranean countries relative to northern European countries.

There is increasing evidence that following a Mediterranean diet correlates with greater longevity and delays the onset of age-associated health deterioration, not only in Mediterranean countries but also in non-Mediterranean countries.[25,26] Although many studies have focused on particular food substances in the Mediterranean diet that are responsible for maintaining human health, the results have not been adequately confirmed. As shown in the previous reports and in Table 1, polyamines are contained in most foods in widely varying concentrations,[12,13] therefore differences in dietary pattern greatly influence the amount of polyamine intake. As expected, most Mediterranean diet food consumption is associated with increased polyamine amount.

Among the mechanisms that account for the protective effect of the Mediterranean diet against age-associated health deterioration is that the Mediterranean diet seems to have a protective effect against mild chronic inflammation and its metabolic complications.[18–20,27,28] Similar to n-3 unsaturated fatty acids, which have been shown to decrease age-associated health deterioration,[29,30] polyamine, especially spermine, has an anti-inflammatory effect by suppressing inflammatory mediators.[16,17] In addition, an increase in intracellular polyamines from extracellular sources seems to help maintain vascular health. The increase in intracellular polyamines suppresses enzymatic activities needed for polyamine synthesis. Because polyamines are synthesized from arginine, this suppression could increase the amount of arginine available for nitric oxide synthesis. Decreased bioavailability of nitric oxide is involved in the pathogenesis of various disorders,[31] and, conversely, increased nitric oxide maintains normal vascular function.[32,33] Therefore, continuously increased polyamine intake from foods increases nitric oxide availability and helps slow the progression of age-associated vascular disorders, which are the biggest killer of adults in European and Western countries.

This is an epidemiologic study, so there may be confounding factor(s) between the amount of polyamine and the Mediterranean diet foods. Moreover, polyamine content in food is affected by the effects of food processing and storage, and one of the weaknesses of the present study was the inability to comprehend these changes.[32,33] However, the present findings, together with previous studies on polyamines,[23] indicate a possible role for the food polyamines that are abundant in the Mediterranean diet in prolonging human life.

Disclosure

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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Lack of an Association between the *PPARG2 Pro12Ala* Polymorphism and Glucose Intolerance in a Vietnamese Population

ベトナム人において *PPARG2 Pro12Ala* 多型と糖代謝異常は関連しない

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Abstract　Single nucleotide polymorphisms of the transcription factor and peroxisome proliferator-activated receptor gamma 2 (*PPARG2*) gene such as *Pro12Ala* (rs1801282) has been reported to decrease the risk of type-2 diabetes (T2D) in multiple populations. However, similar effects have yet to be confirmed across different ethnic populations. This study addressed the question whether *PPARG2 Pro12Ala* decreases the risk of glucose intolerance in the Vietnamese population, where obesity is rare and yet the T2D incidence is increasing. We recruited a total of 173 glucose intolerant subjects and 310 gender and age-group–matched normoglycaemic controls at random. The minor Ala allele frequency of *Pro12Ala* was similarly low in both glucose intolerant subjects and normoglycemic controls (2.9% and 2.4%, respectively) with no significant association between *PPARG2 Pro12Ala* and glucose intolerance. Thus, *PPARG2 Pro12Ala* is not a crucial genetic marker for the prediction of risk of T2D in Vietnamese.

Keywords:　Vietnamese population ベトナム, *PPARG2* ペルオキシソーム増殖因子活性化受容体 γ 2, polymorphism 遺伝子多型, glucose intolerance 糖代謝異常

INTRODUCTION

An enormous number of candidate gene studies, including in-depth studies of genes and genome-wide association studies (GWAS) have been carried out and recently identified quite a few genes which could be linked with the susceptibility to type 2 diabetes (T2D). The gene of transcription factor peroxisome proliferator activated receptor gamma 2 (*PPARG2*) which has been known to play an important role in adipocyte differentiation, insulin action and glucose metabolism is one of such genes [1]. A relatively common

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polymorphism of it, rs1801282 (*Pro12Ala*), a missense mutation in exon 2, resulting in an amino acid change, proline to alanine at codon 12 was initially identified as a protective genetic factor of T2D in Finns and Japanese Americans [2]. The *PPARG2* 12Ala allele improves insulin sensitivity and confers protection against T2D[2-3]. The frequencies of this mutation varied in diverse populations: Caucasians had a highest proportion carrying *PPARG2 Pro12Ala* (12%), followed by Mexican Americans (10%), West Samoans (8%), and African Americans (3%), while Chinese have the lowest (1%) [4]. The effects of this polymorphism on T2D has been confirmed in several GWAS in Caucasians [5], and also in candidate gene studies in multiple populations such as Scottish [6], Russians [7], Indians [8], Chinese [9] and Asian Indian Sikhs [10].

However, the significance of the *PPARG2 Pro12Ala* polymorphism as the anti-risk of T2D is still controversial. Some studies showed no association between this polymorphism and T2D in French [11], Italians [12], Danish Caucasians [13] or South Indians [14]. A growing body of evidence suggests that these inconsistent findings from subsequent studies in different ethnic populations could be partially explained by complex interactions between this polymorphism and environmental factors such as dietary fat composition and/or physical activities [15]. In addition, it has been reported that the preventive effects of this polymorphism on T2D could be mediated by obesity. A study of obese and non-obese Europeans found that the association of the *PPARG2 Pro12Ala* with T2D was more significant in obese individuals (BMI ≥ 30 kg/m^2) [16]. A meta-analysis review by Tonjes et al. also demonstrated that effects of Ala homozygous only appear in obese subjects [17]. Therefore, the significance of this *PPARG2 Pro12Ala* polymorphism with the development of T2D remains to be elucidated in a variety of other ethnic populations with different genetic backgrounds and environmental factors.

Recently, a remarkable increase of the incidence of T2D was noticed in Vietnam, as a consequence of rapid urbanization, demographic changes and westernizing changes in lifestyles and dietary habits. Similarly to other Southeast Asians, Vietnamese are facing with the widespread occurrence of T2D, even with a moderate weight gain and at a younger age as compared with Western populations [18]. While obesity has been considered as a major driving factor for insulin resistance, glucose intolerance and T2D in Caucasians, body composition is thought to contribute to the high susceptibility to T2D among Vietnamese. Central adiposity reflected by high waist: hip ratio (WHR) is observed as a common feature in both non-diabetes and diabetes Vietnamese even though their BMI levels are still within normal range and central adiposity was found to be associated with 2.4-fold increases in the risk of T2D in Vietnamese [19]. Furthermore, westernized dietary and lifestyle changes could be another possible explanation for the increased incidence of T2D in Vietnam. Traditional lifestyles with sufficient physical activity and moderate fat intake in the past were recently changed into sedentary lifestyles, unhealthy dietary practices such as excess energy intake, high protein intake, high consumption of saturated fats, and refined carbohydrates which have the high glycemic index (GI) values. It is known that the chronic consumption of high GI foods results in huge fluctuations in blood glucose and insulin levels, therefore worsen insulin resistance in susceptible populations [20]. In addition, dyslipidemia, another common feature in Vietnamese, even among non-obese subjects (unpublished data), could be linked to the pathogenesis of insulin resistance and T2D in Vietnamese. Therefore, genetic background, particularly gene involving in adiposity and lipid metabolism is thought to be responsible for the high susceptibility to T2D in Vietnamese. Based on the aforementioned linkage of *PPARG2* with adipocyte differentiation, adipogenesis, insulin action and glucose metabolism, and the need to clarify the effect of rs1801282 polymorphism on the development of T2D in various populations, the present study addressed the question whether the *PPARG2* rs1801282 polymorphism decreases the risk of glucose intolerance in a Vietnamese population.

METHODS

Study design and measurements

The present study is derived from the population-based, cross-sectional epidemiological study in Ho Chi Minh City, Vietnam, which was designed to investigate the prevalence of metabolic syndrome and T2D among a population aged 30-69 years. The study was approved by Ethical and Scientific Research Board of Ho Chi Minh City Nutrition Center, Vietnam and received written informed consent from each participant. All study subjects underwent a detailed clinical examination, including measurements of weight, height, waist circumference and blood pressure, sampling of venous blood at 5:00 a.m. – 7:00 a.m. after overnight fasting and 75-g oral glucose tolerance test (OGTT). Body weight and height were measured in light clothes without shoes to the nearest 0.1 kg for weight and 0.5 cm for height. Waist circumference was measured at the midpoint between the lowest rib and the iliac crest to the nearest 0.1 cm using an inelastic tape. Blood pressure was measured twice on the left arm of the participant in the sitting position after 5 min of rest. Body mass index (BMI) was determined as weight in kilograms divided by the square of the height in meters (kg/m^2). Fasting serum was separated from coagulated whole blood and insulin, total cholesterol, high-density lipoprotein cholesterol (HDL-C) and triglycerides were measured by Diag Center International (Lab Group International - Division Vietnam). The homeostasis model of assessment of insulin resistance (HOMA-IR) score was calculated as fasting insulin (μU/ml) multiplies by fasting glucose (mmol/l) divided by 22.5 [21]. Fasting glucose and 2-h postload glucose levels of 75-g OGTT were used to stratify subjects into 4 groups according to the 1999 World Health Organization criteria: normal glucose tolerance (NGT); impaired fasting glucose (IFG) ; impaired glucose tolerance (IGT) and type 2 diabetes (T2D) [22]: for T2D. fasting $\geq$ 7.0 mmol/l (126 mg/dl), 2-h $\geq$ 11.1 mmol/l (200 mg/dl): for IGT, fasting < 7.0 mmol/l (126 mg/dl) and 2-h $\geq$ 7.8 mmol/l (140 mg/dl) and < 11.1 mmol/l (200 mg/dl); and for IFG $\geq$ 6.1 mmol/l (110 mg/dl) and < 7.0 mmol/l (< 126 mg/dl) and if measured, 2-h < 7.8 mmol/l (140 mg/dl). A total of 173 glucose intolerant (IGT+T2D) subjects and 310 gender and age-group–matched normoglycaemic (NGT) controls were randomly recruited.

SNP genotyping

Genomic DNA was isolated from frozen EDTA-anticoagulated whole blood specimens using Gentra Puregene Blood Kit (QIAGEN Inc, Valencia, CA, USA). The concentration and quality of DNA were determined by NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Genomic DNA isolated from whole blood was genotyped using a TaqMan Drug Metabolism Assay with allele-specific probes (assay ID C_1129864_10; Applied Biosystems Inc., Foster City, CA, USA). Briefly, genomic DNA (20 ng), TaqMan Genotyping Master Mix, pair of primers, and each allele-specific TaqMan probe labeled with FAM or VIC were added to 96-well plates. Real-time PCR and genotyping based on the fluorescence intensities of 2 dyes were performed by ABI Prism 7900 HT Sequence Detection System (Applied Biosystems Inc., Foster City, CA, USA) according to the manufacturer' s instruction. The authenticity of genotyping was validated by sequencing amplicons of PCR performed on a set of randomly chosen genomic DNA samples containing common homozygous, heterozygous and rare homozygous genotypes that were determined by TaqMan genotyping assay. As quality control, known negative and positive control samples were used and run in parallel with unknown DNA samples. 5% of samples were randomly selected and re-genotyped to assess the reproducibility of genotyping. SNP genotyping was performed in the Cardiovascular Research Institute, Saitama Medical Center, Jichi Medical University, Saitama, Japan.

Statistical analysis

Data were analyzed by using the statistical package SPSS for Windows (SPSS Inc., Chicago, IL, USA). Calculate prevalence with 95% confidence intervals for categorical variables and mean with 95% confidence intervals for continuous variables. Differences in average levels of biological markers among subgroups were examined using one way ANOVA and t-tests for parametrically distributed variables and nonparametric Kruskal Wallis and Mann-Whitney U tests for nonparametrically distributed variables. Differences in genotype and allele frequencies between glucose tolerant cases and normoglycemic controls were examined using χ^2 test or Fisher exact probability test when expected cell values were less than 3. A level of $P < 0.05$ is considered statistically significant.

RESULTS

Characteristics of 2 study groups

The characteristics of glucose intolerant and normoglycemic groups are shown in Table I. Two groups were similar in gender distributions, whereas glucose intolerant subjects were slightly older than normal controls ($P < 0.05$). Glucose intolerant subjects had significantly greater BMI, higher triglyceride, fasting glucose and fasting insulin levels and greater HOMA-IR score but had significantly lower HDL-C levels (all P values < 0.001) than normal controls. There was no significant difference between 2 groups in waist circumference, blood pressure or total cholesterol levels.

The frequency of *PPARG2 Pro12Ala* genotypes and the minor Ala allele in glucose intolerant and normoglycemic subjects

The frequency of common homozygous Pro/Pro, heterozygous Pro/Ala and rare homozygous Ala/Ala genotypes at the codon 12 of *PPARG2* in glucose intolerant subjects was 95.4%, 3.5% and 1.2%, respectively whereas that in normoglycemic subjects was 95.2, 4.8% and 0.0%, respectively (Table II). The minor Ala-allele frequency in glucose intolerant

Table I　Characteristics of 2 study groups

Characteristics	Normal subjects (n = 310)	Glucose intolerant subjects (n = 173)
Gender, {n (%)}		
Men	131 (65.2)	70 (34.8)
Women	179 (63.5)	103 (36.5)
Average age (yrs)	53.8 ± 8.9	55.8 ± 8.7*
BMI (kg/m^2)	23.1 ± 3.2	24.3 ± 3.8**
Waist circumference (cm)	79.8 ± 9.5	80.4 ± 9.2
Systolic BP (mmHg)	120.5 ± 19.8	122.7 ± 21.8
Diastolic BP (mmHg)	74.3 ± 11.7	74.3 ± 11.1
Total cholesterol (mg/dl)	209.7 ± 42.5	210.3 ± 52.5
HDL-C (mg/dl)	54.7 ± 13.7	51.3 ± 13.3***
Triglyceride (mg/dl)	146.0 (125.5)	179.0 (144.5)***
Fasting glucose (mmol/l)	5.1 (0.4)	5.8 (1.0)***
Fasting insulin (μU/ml)	5.6 (5.9)	10.2 (9.7)***
HOMA-IR	1.3 (1.3)	2.8 (2.4)***

Average age, BMI, waist circumference, body fat percentage, total cholesterol and HDL-C are expressed as the mean ± SD and differences among groups were examined by one way ANOVA and t-test. Triglyceride, fasting glucose, fasting insulin and HOMA-IR are presented as median followed by interquartile range in parentheses and differences among groups were examined by non-parametric Mann-Whitney test. P values refer to differences as determined by t tests or Mann-Whitney test.

* $P < 0.05$　**$P < 0.01$　*** $P < 0.001$, compared with normal controls

Table II Genotype and allele frequencies of *PPARG2 Pro12Ala*

	Normal subjects	Glucose intolerant subjects
Genotype		
Wild type (Pro12Pro)	295 (95.2%)	165 (95.4%)
Heterozygous (Pro12Ala)	15 (4.8%)	6 (3.5%)
Homozygous (Ala12Ala)	0 (0.0%)	2 (1.2%)
Total	310	173
Allele		
Ala allele	15 (2.4%)	10 (2.9%)
Pro allele	605 (97.6%)	336 (97.1%)
Total	620	346

Data represent number (frequency). Differences in genotype and allele frequencies between glucose tolerant cases and normoglycemic controls were examined using χ^2 test or Fisher exact probability test when expected cell values were less than 3. No significant difference between two groups in the frequency of the genotypes and minor Ala allele was found (all P values > 0.05).

and normoglycemic subjects was 2.9% and 2.4%, respectively. There was no significant difference between 2 groups in the frequency of the genotypes and minor Ala allele.

DISCUSSION

To our knowledge, this study was the first to investigate the association between the *PPARG2* polymorphism and glucose intolerance in a Vietnamese population. The frequency of Ala allele was 2.9% and 2.4% in glucose intolerant subjects and normal controls, respectively. We found no significant difference in the frequency of common homozygous *Pro12Pro*, heterozygous Pro12Ala and rare homozygous Ala12Ala genotypes at *PPARG2 Pro12Ala* between glucose intolerant subjects and normoglycemic controls.

Since the frequencies of the *PPARG2* Ala allele in glucose intolerant subjects and normal controls were very similar, a similar Ala-allele frequency would be expected in a general Vietnamese population. The frequency of the *PPARG2* Ala allele was quite low in the Vietnamese population compared with Caucasians, but comparable with that from African Americans and Japanese [4, 23].

The lack of association between the *PPARG2 Pro12Ala* variant and glucose intolerance was partly in agreement with a previous report in Swedish population showing that the incidence of T2D and IGT was comparable in the three *PPARG2 Pro12Ala* genotypes [24]. Similarly, Rahda *et al.* indicated the lack of association of the *PPARG2 Pro12Ala* with T2D and insulin sensitivity in South Asians [25].

Several possibilities might potentially be involved in the lack of the effect of the *PPARG2 Pro12Ala* on glucose intolerance. 1) Obesity, an important factor that promotes glucose intolerance and insulin resistance, might mediate the association between the *PPARG2 Pro12Ala* variant and glucose intolerance. The population subjected to the present study consists of relatively few obese people. In support of this hypothesis, the significant association between T2D and the *PPARG2 Pro12Ala* variant was modified by BMI [26] or was detected in only obese subjects but not in non-obese subjects [16]. Furthermore, Tonjes *et al.* reported that greater insulin sensitivity of the Ala-allele carriers was only found in obese individuals [17]. 2) Interactions with other genes or environmental factors might contribute to the disparate effect of this *PPARG2 Pro12Ala* polymorphism on the development of T2D. Previous studies showed that this polymorphism alone did not show a positive effect on T2D, but with the presence of other polymorphisms within the *PPARG2* gene such as

C1431T [6] or -1279G/A and His478His variants [14], the positive association of the *PPARG2 Pro12Ala* with T2D was found. In addition, some reports showed that effects of effect of the *PPARG2 Pro12Ala* polymorphism on the body weight and insulin sensitivity could be modified by dietary fatty acids or physical activity [15]. Variations in dietary intake of polyunsaturated fat versus saturated fat appeared to influence BMI and fasting insulin in Ala variant carriers [27]. Moreover, dietary intake of monounsaturated fatty acids was found to be inversely associated with insulin resistance in Ala allele carriers [28]. In the Quebec Family Study, Pro homozygous had lower BMI, waist circumference and fat mass (both subcutaneous and visceral) at baseline, but responded to an increase in dietary fat with a gradual increase in BMI and waist circumference, whereas those effects were not observed in Ala allele carriers [29]. Recently, Ruchat et al. reported that Ala allele carriers appear to be more responsive to regular endurance training in improving glucose metabolism and insulin sensitivity compared with Pro homozygous [30]. Studies in different Indian ethnicities revealed an extensive diversity of the association between the *PPARG2 Pro12Ala* and T2D, further supporting the hypothesis of gene-environment interactions. The *PPARG2 Pro12Ala* polymorphism was a strong predictor for T2D in Indian Sikhs [10] but not in South Indians from Chennai [31].

In order to clarify these above questions, the larger sample size may be necessary. Because even the previous findings claiming the association of this polymorphisms with T2D showed that homozygous for the higher risk Pro allele attributes a weak effect on T2D (only a 25% increase in diabetes risk) [3], and we revealed in the present study that the Ala-allele frequency in Vietnamese was quite low.

In conclusion, we found a low frequency of the Ala allele at the *PPARG2 Pro12Ala* and the lack of association between the *PPARG2 Pro12Ala* polymorphism and glucose intolerance in a Vietnamese population. Thus, this polymorphism is not a crucial genetic marker that predicts subjects susceptible to glucose intolerance at least in Vietnamese population with moderate BMI.

〔要　約〕

転写因子ペルオキシソーム増殖因子活性化受容体γ2（*PPARG2*）の一塩基多型である *Pro12Ala*（rs1801282）変異は，2型糖尿病のリスク軽減に関連すると多くの集団で報告されてきたが，人種によっては同様の結果が得られていない。本研究は，肥満が少ないにもかかわらず2型糖尿病が増加しているベトナム人において *PPARG2 Pro12Ala* 多型が糖代謝異常の危険因子であるかを検討することを目的とした。対象は無作為に糖代謝異常者173名とこれに性・年齢をマッチさせた310名の対照者を選んだ。*Pro12Ala* アリル頻度は，糖代謝異常者群2.9%と対照者群2.4%でともに低く，糖代謝異常と *PPARG2 Pro12Ala* 多型との有意な関連は認められなかった。すなわち，*PPARG2 Pro12Ala* 多型はベトナム人における2型糖尿病リスク予測のための確定的遺伝マーカーではない。

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