発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Mulu A, Kassu A, Huruy K, Tegene B, Yitayaw G, Nakamori M, Van Nhien N, Bekele A, Wondimhun Y, Yamamoto S, Ota F.	Vitamin A deficiency during pregnancy of HIV infected and non-infected women in tropical settings of Northwest Ethiopia.	BMC Public Health.	15;11	569	2011
Sarukura N, Kogirima M, Takai S, Kitamura Y, Kalubi B, Yamamoto S, Takeda N.	Dietary zinc intake and its effects on zinc nutrition in healthy Japanese living in the central area of Japan.	J Med Invest.	58(3-4)	203-9	2011
Yamaguchi A, Tanaka N, Eguchi Y, Kuno K, Wakikawa N, Sarukura N, Fukinbara M, Yamamoto S.	Study on the necessary survey days for energy intake in school children assessed by 7 day survey.	J Med Invest.	59(1-2)	111-5	2012
島野仁					
Totsuka K, Maeno T, Saito K, Kodama S, Asumi M, Yachi Y, Hiranuma Y, Shimano H, Yamada N, Ono Y, Naito T, Sone H.	Self-reported fast eating is a potent predictor of development of impaired glucose tolerance in Japanese men and women: Tsukuba Medical Center Study.	Diabetes Res Clin Pract	94	e72-4	2011
Saito R, Matsuzaka T, Karasawa T, Sekiya M, Okada N, Igarashi M, Matsumori R, Ishii K, Nakagawa Y, Iwasaki H, Kobayashi K, Yatoh S, Takahashi A, Sone H, Suzuki H, Yahagi N, Yamada N, Shimano H.	Macrophage Elovl6 deficiency ameliorates foam cell formation and reduces atherosclerosis in low-density lipoprotein receptor-deficient mice.	Arterioscler Thromb Vasc Biol.	31	1973-79	2011
Heianza Y, Hara S, Arase Y, Saito K, Fujiwara K, Tsuji H, Kodama S, Hsieh SD, Mori Y, Shimano H, Yamada N, Kosaka K, Sone H.	HbA_{1c} 5.7-6.4% and impaired fasting plasma glucose for diagnosis of prediabetes and risk of progression to diabetes in Japan (TOPICS 3): a longitudinal cohort study.	Lancet	378	147-155	2011

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
山下静也					
Hanada H, Nishida M, Yamashita S. et al.	Establishment of chemiluminescence enzyme immunoassay for apolipoprotein B-48 and its clinical applications for evaluation of impaired chylomicron remnant metabolism	Clin Chim Acta	413	160-165	2012
Masuda D, Nishida M, Yamashita S.et al.	Fasting serum apolipoprotein B-48 level can be a marker of postprandial hyperlipidemia	J Atheroscler Thromb	18	1062-1070	2011
Nakatani K, Nishida M, Yamashita S.et al.	Serum apolipoprotein B-48 levels are correlated with carotid intima-media thickness in subjects with normal serum triglyceride levels	Atherosclerosis	218(1)	226-232	2011
船橋徹					
Kishida K, Kim KK, Funahashi T, Matsuzawa Y, Kang HC, Shimomura I	Relationships between Circulating Adiponectin Levels and Fat Distribution in Obese Subjects	J Atheroscler Thromb.	18	592-595	2011
Nakagawa Y, Kishida K, Kihara S, Yoshida R, Funahashi T, Shimomura I	Nocturnal Falls of Adiponectin Levels in Sleep Apnea with Abdominal Obesity and Inpact of Hypoxia-Induced Dysregulated Adiponectin Production in Obese Murine Mesenteric Adipose Tissue.	J Atheroscler Thromb	18	240-247	2011

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Nakagawa Y, Kishida K, Mazaki T, Yokoi H, Nobuyoshi M, Funahashi T,Shimomura I.	Inpact of Sleep-Disordered Breathing, Visceral Fat Accumulation and Adiponectin Levels in Patients with Night-Time Onset of Acute Coronary Syndrome.	Am J Cardiol	108(9)	1266-71	2011
石川鎮清					
Tsutsumi A, Kayaba K, Ishikawa S.	Impact of occupational stress on stroke across occupational classes and genders.	Soc Sci Med	72	1652-8	2011
Shibata Y, Hayasaka S, Yamada T, Ojima T, Ishikawa S, Kayaba K, Gotoh T, Nakamura Y.	Physical activity and risk of fatal or non-fatal cardiovascular disease among CVD survivors: the JMS cohort study.	Circ J	75	1368-72	2011
Nago N, Ishikawa S, Goto T, Kayaba K. 5.	Low cholesterol is associated with mortality from stroke, heart disease, and cancer: the Jichi Medical School Cohort Study.	J Epidemiol	21(1)	67－74	2011
Yamada T, Hayasaka S, Shibata Y, Ojima T, Saegusa T, Gotoh T, Ishikawa S, Nakamura Y, Kayaba K.	Frequency of citrus fruit intake is associated with the incidence of cardiovascular disease: the Jichi Medical School cohort study.	J Epidemiol	21(3)	169-175	2011
Iwahana H, Ishikawa S, Ishikawa J, Kabutoya T, Kayaba K, Gotoh T, Kajii E.	Atrial fibrillation is a major risk factor for stroke, especially in women: the Jichi Medical School cohort study.	J Epidemiol	21(2)	95-101	2011
河野幹彦					
Binh PNT.,Abe, Y., Tien PG., Son LNTD., Hanh TT., Diep DTN., Qui LTK., Kawano M., Murayama C.	Plasma NOx concentrations in glucose intolerance and type 2 diabetes: a case-control study in a Vietnamese population.	J Atheroscl Thromb	18(4)	305-311	2011

Effects of Topical Nipradilol on Early Diabetic Retinopathy in SDT Rats

Nozomi Kinoshita[1], Akihiro Kakehashi[*,1], Yoh Dobashi[1], Ryuichiro Ono[1], Fumihiko Toyoda[1], Chiho Kambara[1], Hiroko Yamagami[1], Yusuke Kitazume[2], Eiji Kobayashi[3], Yasuhiro Osakabe[4], Motoshige Kudo[4], Masanobu Kawakami[1] and Yasunori Kanazawa[5]

[1]*Saitama Medical Center, Jichi Medical University, Saitama, Japan*

[2]*Medical Affairs Department, Kowa Pharmaceutical Company, Ltd., Japan*

[3]*Center of Molecular Medicine, Jichi Medical University, Shimotsuke, Japan*

[4]*Department of Pathology, Tokyo Medical University, Tokyo, Japan*

[5]*Japanese Diabetes Foundation, Tokyo, Japan*

Abstract: We tested the effect of topical nipradilol, an antiglaucoma drug with α- and β-blocker and nitric oxide (NO) donor activities, on the early retinal changes using electron microscopy in Spontaneously Diabetic Torii (SDT) rats. In seven young male SDT rats (17 to 18 weeks old), nipradilol solution was instilled in the right eyes and nipradilol-free base solution in the left eyes three times daily for 3 months. All rats were sacrificed and both eyes were enucleated for electron microscopy at the end of the experiments. All rats had blood glucose levels exceeding 350 mg/dl within 2 weeks after the beginning of the experiment (mean final blood glucose level, 558±100.2 mg/dl). In untreated eyes of young SDT rats, the overall pathological features were almost comparable to those in older SDT rats, although the pinocytotic vesicles and free ribosomes were not as remarkable in the latter. In contrast, in nipradilol-treated eyes of SDT rats, although the basement membrane was thickened, microvilli were seen, and a larger number of electron-dense mitochondria were in the wide cytoplasm. Lipofuscin-like electron-dense granules and lamellated myelin figures also were identified. Nipradilol reduced the early morphologic changes in the endothelial cells, which reflects the metabolically active state of diabetic retinopathy in SDT rats through its action as a NO donor.

Keywords: Nipradilol, α- and β-blocker, nitric oxide (NO) donor, SDT rat, diabetic retinopathy, electron microscopy.

INTRODUCTION

The Spontaneously Diabetic Torii (SDT) rat is a novel animal model of proliferative diabetic retinopathy (DR). Large retinal folds of thickened retina with vitreous traction and extensive dye leakage around the optic disc combined with vascular tortuosity by fluorescein microangiography are the most typical characteristics of advanced retinopathy in SDT rats (Fig. **1**). The prevalence of advanced retinopathy in SDT rats is about 80% by 60 weeks of age, but advanced retinopathy is rarely seen before 50 weeks of age [1]. Microscopic changes of DR can be observed much earlier than those macroscopic changes. Microscopic changes of DR in old SDT rats include basement membrane (BM) thickening in the form of reduplication caused by invagination of the cytoplasmic process into the membrane. Furthermore, in the endothelial cells, clear mitochondria and dilated rough endoplasmic reticulum are abundant. Those findings are not observed in old normal non-diabetic SD rats. Thus, these morphologic changes are not age-related but caused by diabetes (Fig. **2**) [2].

*Address correspondence to this author at the Department of Ophthalmology, Saitama Medical Center, Jichi Medical University, 1-847 Amanuma, Omiya, Saitama, 330-8503, Japan; Tel: +81-48-647-2111; Fax: +81-48-648-5188; E-mail: kakeaki@omiya.jichi.ac.jp

In early-stage DR, electrophysiologic changes such as the absence of oscillatory waves in the electroretinogram are seen even with no funduscopic changes. Previous studies showed that leukocyte adhesion to the retinal endothelial cells initiated DR [3-6]. Using acridine orange leukocyte digital fluorography, we previously reported that topical nipradilol, an antiglaucoma agent with α- and β-blocker and nitric oxide (NO) donor activity (3,4-dihydro-8-(2-hydroxy-3-isopro-pylamino) propoxy-3-nitroxy-2*H*-1-benzo-pyran; molecular weight, 326.34) (Fig. **3**) significantly reduced leukocyte adhesion to the endothelium of retinal vessels in early-phase DR in streptozotocin (STZ)-induced diabetic rats [7].

In the current study, we tested the effect of nipradilol on early retinal changes in SDT rats using electron microscopy.

METHODS

All procedures were performed according to the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research and approved by the Jichi Medical University Animal Care and Use Committee.

Animals

Seven young male SDT rats (17 to 18 weeks old, CLEA, Inc., Tokyo, Japan) were used. Nipradilol 1% ophthalmic

1876-5246/11

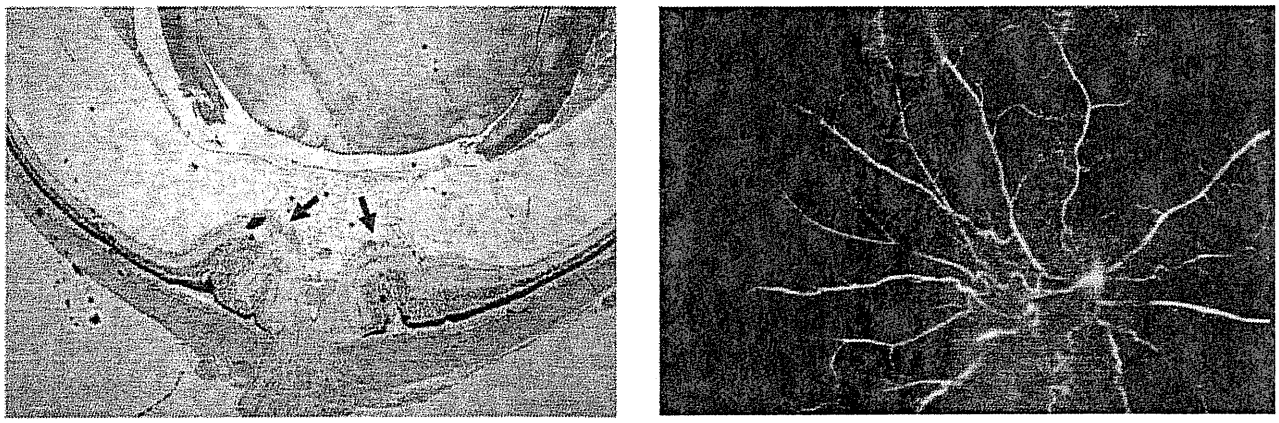

Fig. (1). Proliferative DR in SDT rats. Large retinal folds of thickened retina with vitreous traction (arrows) are the most typical characteristics of advanced retinopathy (left). Fluorescein microangiography shows extensive dye leakage around the optic disc with vascular tortuosity (right) (modified from Kakehashi [2]) in SDT rats over 50 weeks of age.

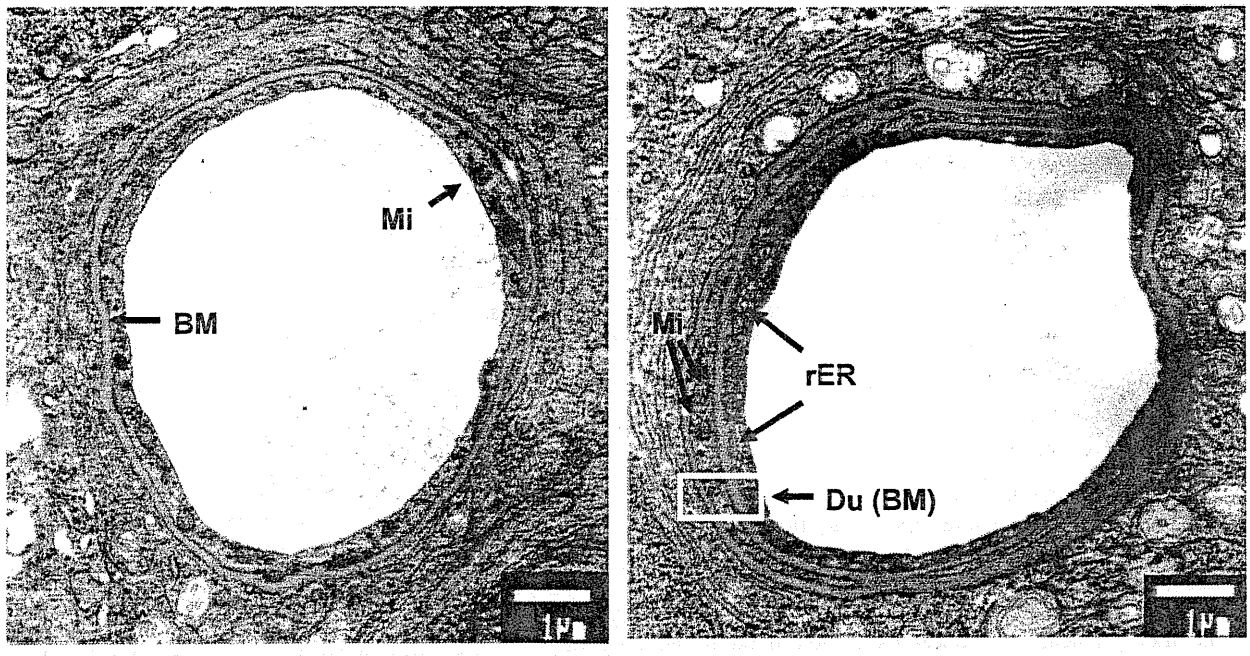

Fig. (2). Electron microscopic findings in the retinal capillary in older SD rats and SDT rats (modified from Kakehashi [2]). Basement membrane (BM) thickening by invagination of the endothelial cytoplasm is prominent in an older untreated SDT rat (right) compared with the thin clean membrane in a normal SD rat (left). Clear mitochondria (Mi) and dilated rough endoplasmic reticulum (rER) and reduplication (Du) of the BM are seen in an older untreated SDT rat (right).

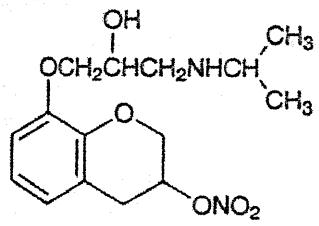

Fig. (3). Nipradilol. Nipradilol (3,4-dihydro-8-(2-hydroxy-3-isopropylamino)propoxy-3-nitroxy-2*H*-1-benzopyran; molecular weight, 326.34) has not only antiglaucomatous effects that depend mainly on selective α1-receptor and nonselective β-receptor blocking activity to reduce intraocular pressure but also NO donor activity through its nitroxyl group.

solution (Kowa Pharmaceutical Company, Ltd., Japan) was instilled in the right eyes and nipradilol-free base solution was instilled in the control left eyes three times daily for 3 months. Blood glucose levels were measured at baseline, 2 weeks, and 1, 2, and 3 months after the start of treatment.

Procedure to Observe Early Retinal Changes in SDT Rats

All rats were sacrificed and both eyes were enucleated about 3 months after the start of the experiment (about 28 weeks of age). We performed electron microscopy to compare the changes in retinal vasculature between nipradilol-treated eyes and untreated control eyes in the SDT rats. The anesthetized SDT rats were perfused via the left

ventricle with 300 ml of 2.5% glutaraldehyde in 0.2 M phosphate buffer (pH, 7.4). The retina was dissected from the eye cup and post-fixed in phosphate buffered 1% osmium tetroxide (pH, 7.4), dehydrated in ascending concentrations of ethanol, and passed through n-butyl glycidyl ether. The retinal blocks were embedded in epoxy resin. Thin sections were cut and stained in uranyl and lead salt solutions and observed by electron microscopy (JEM-1200EX, JEOL Ltd., Tokyo, Japan).

RESULTS

All rats were confirmed to have blood glucose levels exceeding 350 mg/dl within 2 weeks after the beginning of the experiment. The mean final blood glucose level 3 months after the beginning of the experiment was 558±100.2 mg/dl.

When we observed retinal specimens from nipradilol-treated and nipradilol-untreated eyes using electron microscopy, the retinal capillaries had well-preserved ultrastructural features and many features of the endothelial cells, including the plasma membrane and basal lamina, could be identified.

In untreated eyes of young SDT rats (Fig. **4**), BM thickening was apparent despite the younger age, and the morphology was almost identical to that of the older SDT rats (Fig. **2**). Although the endothelial cells were plump, the cytoplasmic space was smaller compared with the older non-diabetic SD rats. In this relatively narrow cytoplasmic space, pinocytotic vesicles, clear mitochondria, dilated rough endoplasmic reticulum, and free ribosomes were remarkable. Moreover, pericytes were generally shrunken and decreased in number. These overall features were almost comparable to those in older SDT rats, although the pinocytotic vesicles and free ribosomes were not as remarkable in the latter.

In contrast, in nipradilol-treated eyes of SDT rats (Fig. **5**), the cytoplasmic space in the endothelial cells was broader despite the flattened morphology compared with those in untreated eyes (Fig. **4**). Thus, the cytoplasmic organelles were seen more clearly. Although the pinocytotic vesicles were not abundant, microvilli were observed. The mitochondria were electron-dense, and the numbers were greater compared with those in untreated eyes. Another difference was the electron-dense granules, some of which were reminiscent of lipofuscin. Lamellated myelin figures also were seen. In the nipradilol-treated eyes, there were more and larger pericytes. The alterations of the BM were similarly observed, and no significant difference was found compared with those in the untreated eyes.

DISCUSSION

In early-stage DR, adhesion of leukocytes to the retinal endothelial cells (retinal leukostasis) is considered a key pathogenic process [3-7]. Furthermore, recent studies have suggested that vascular endothelial growth factor (VEGF) levels are increased not only in late-stage [8, 9] but also in early-stage DR [10]. As a result of VEGF-induced leukocyte adhesion to the diabetic retinal vasculature, retinal vascular endothelial cells are affected by leukocytes, resulting in breakdown of the blood-retinal barrier and capillary nonperfusion [11]. In the alloxan-induced diabetic rat,

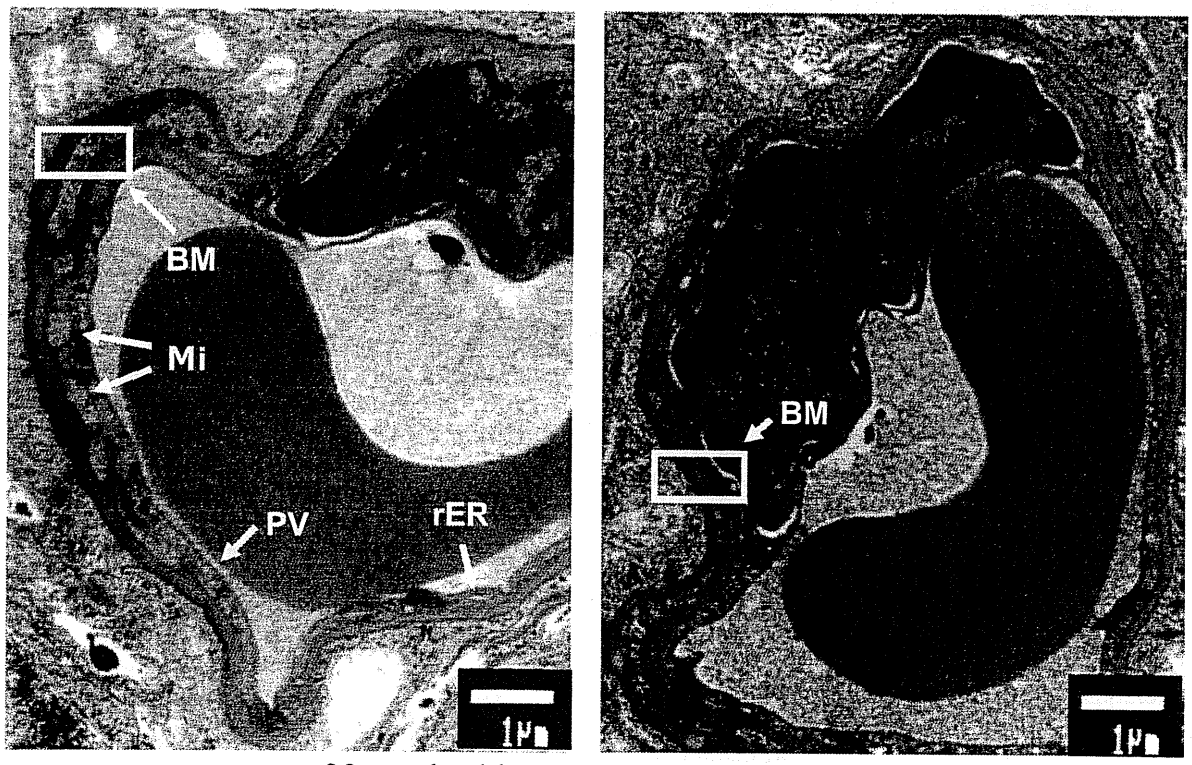

Fig. (4). Electron microscopic findings of the retinal capillaries in a young untreated SDT rat. In the untreated group, BM thickening is apparent despite younger age. The endothelial cells of the retinal capillaries are plump but have narrow cytoplasm (right and left). Pinocytotic vesicles (PV), clear mitochondria (Mi), dilated rough endoplasmic reticulum (rER), and free ribosomes are remarkable (left).

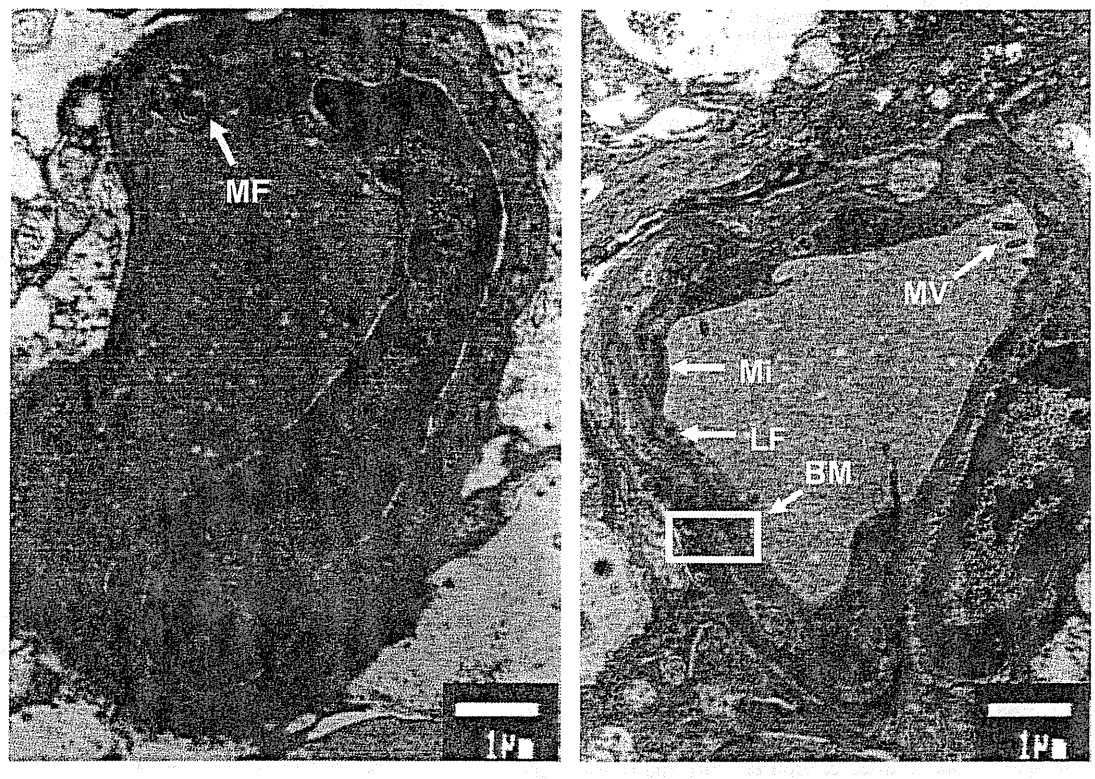

Fig. (5). Electron microscopic findings of the retinal capillaries in a young nipradilol-treated SDT rat. In the nipradilol-treated group, the endothelial cells in the retinal capillaries are flattened but have larger cytoplasm. Microvilli (MV) and electron-dense mitochondria (Mi) are seen (right). Electron-dense lipofuscin (LF)-like granules (right) and lamellated myelin figures (MF) (left) also are seen. The alterations of the basement membrane (BM) were similarly observed compared with those in the untreated eyes (left).

Schroder *et al.* reported that neutrophils and monocytes occluded retinal capillaries in histologic sections and that areas of endothelial cell damage were present around the leukocytes [12]. Therefore, preventing retinal leukostasis may be a possible therapeutic approach [6].

Both reduced retinal blood flow and vasoconstriction of retinal vessel progress in early-stage DR [13]. The important roles of NO in the retinal microcirculation, i.e., increased blood flow through nitroglycerine-like vasodilation and decreased microvascular occlusion through suppression of platelet coagulation and leukocyte adhesion to the endothelium, are well recognized [14]. Nipradilol works as a NO donor agent through its nitroxyl moiety in the molecule in addition to α- and β-blocking effects (Fig. **3**). We reported that topical nipradilol significantly suppressed retinal leukostasis [7], suggesting that the NO derived from nipradilol inhibits leukocyte adhesion to the vascular endothelium.

It is well known that pericyte loss, BM thickening, and proliferation of endothelial cells occur in the retinal capillaries of STZ-induced diabetic rats and mice [15-17] and humans with diabetes [18]. However, the initial changes in early DR are still uncertain. The endothelial changes and subsequent changes in the BM seem to be the initial changes in early DR in SDT rats.

In the current study, pathological changes were observed in the BM, endothelial cells, and pericytes in SDT rats, which are uncommon findings in old non-diabetic SD rats, indicating that those findings are diabetes-related rather than age-related. Moreover, since BM changes also were seen in the nipradilol-treated group, morphologic BM changes seem to be established in early-phase diabetes and cannot be prevented. We also showed that the endothelial cells of the retinal capillaries were plump with smaller cytoplasm in untreated eyes. Furthermore, the ultrastructural findings, such as abundant pinocytotic vesicles, dilated rough endoplasmic reticulum, and free ribosomes reflect the metabolically active state of the endothelial cells. The decrease in the number of pericytes was consistent with a previous study [18]. Among these several findings, abundant pinocytotic vesicles and free ribosomes were more conspicuous. In turn, in nipradilol-treated eyes, the microvilli were preserved and electron-dense granules and presumably lipofuscin were prominent. The coexistence of lamellated myelin figures, often observed as lipofuscin derivatives, supports this. Considering the other findings, including fewer dilated rough endoplasmic reticulum, pinocytotic vesicles, and free ribosomes, these overall results suggested that the metabolic activity elicited and up-regulated by diabetes was suppressed after nipradilol treatment, and nipradilol may further cause "wear-and-tear changes" represented by the appearance of lipofuscin. Thus, endothelial cells may not functionally revert, but their proliferative capacity definitely is down-regulated by topical nipradilol, since these pathological changes in the

endothelial cells decreased significantly in the treated eyes. These findings also may suggest that the NO activity of nipradilol protects the vascular endothelial cells by suppressing retinal leukostasis.

Mizuno *et al.* reported that nipradilol reaches the periocular tissues around the optic disc in rabbit eyes at an effective concentration 60 minutes after topical instillation [19]. Geroski and Edelhauser reported that nipradilol may reach the retina-choroid through the periocular transscleral route, since the molecular weight of nipradilol is as small as 326.34 [20, 21]. Therefore, topical nipradilol may contribute NO to the retinal circulation.

CONCLUSION

In conclusion, the pathological changes of the BM in DR are established in the early phase and treatment with topical nipradilol did not prevent them. However, nipradilol reduced the early morphologic changes in the endothelial cells, which reflects the metabolically active state of DR in SDT rats. Therefore, this drug may have prophylactically prevented development of proliferative changes in the endothelial cells in DR through its activity as a NO donor. In the future, our next experimental work will clarify whether nipradilol can prevent advanced DR or not.

REFERENCES

[1] Kakehashi A, Saito Y, Mori K, *et al.* Characteristics of diabetic retinopathy in SDT rats. Diabetes Metab Res Rev 2006; 22: 455-61.

[2] Kakehashi A. Pathogenesis of diabetic retinopathy: Lessons from diabetic animal model. Endocrinol Diabetol 2003; 17:31-7 (Japanese).

[3] Abiko T, Abiko A, Clermont AC, *et al.* Characterization of retinal leukostasis and hemodynamics in insulin resistance and diabetes: role of oxidants and protein kinase-C activation. Diabetes 2003; 52: 829-37.

[4] Kinoshita N, Kakehashi A, Inoda S, *et al.* Effective and selective prevention of retinal leukostasis in streptozotocin-induced diabetic rats using gliclazide. Diabetologia 2002; 45: 735-9.

[5] Miyamoto K, Hiroshiba N, Tsujikawa A, Ogura Y. *In vivo* demonstration of increased leukocyte entrapment in retinal microcirculation of diabetic rats. Invest Ophthalmol Vis Sci 1998; 39: 2190-4.

[6] Miyamoto K, Khosrof S, Bursell SE, *et al.* Prevention of leukostasis and vascular leakage in streptozotocin-induced diabetic retinopathy *via* intercellular adhesion molecule-1 inhibition. Proc Natl Acad Sci U S A 1999; 96: 10836-41.

[7] Ono R, Kakehashi A, Ito Y, *et al.* Effect of topical nipradilol on retinal microvascular leukocyte adhesion in diabetic rats. Ophthalmic Res 2006; 38: 270-3.

[8] Adamis AP, Miller JW, Bernal MT, *et al.* Increased vascular endothelial growth factor levels in the vitreous of eyes with proliferative diabetic retinopathy. Am J Ophthalmol 199415; 118: 445-50.

[9] Aiello LP, Avery RL, Arrigg PG, *et al.* Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. N Engl J Med 1994; 331: 1480-7.

[10] Tanaka Y, Katoh S, Hori S, Miura M, Yamashita H. Vascular endothelial growth factor in diabetic retinopathy. Lancet 1997; 349(9064): 1520.

[11] Qaum T, Xu Q, Joussen AM, *et al.* VEGF-initiated blood-retinal barrier breakdown in early diabetes. Invest Ophthalmol Vis Sci 2001; 42: 2408-13.

[12] Schroder S, Palinski W, Schmid-Schonbein GW. Activated monocytes and granulocytes, capillary nonperfusion, and neovascularization in diabetic retinopathy. Am J Pathol 1991; 139: 81-100.

[13] Wright WS, Harris NR. Ozagrel attenuates early streptozotocin-induced constriction of arterioles in the mouse retina. Exp Eye Res 2008; 86: 528-36.

[14] Dorner GT, Garhofer G, Kiss B, *et al.* Nitric oxide regulates retinal vascular tone in humans. Am J Physiol Heart Circ Physiol 2003; 285: H631-6.

[15] Kato N, Yashima S, Suzuki T, Nakayama Y, Jomori T. Long-term treatment with fidarestat suppresses the development of diabetic retinopathy in STZ-induced diabetic rats. J Diabetes Complications 2003; 17: 374-9.

[16] Song E, Dong Y, Han LN, *et al.* Diabetic retinopathy: VEGF, bFGF and retinal vascular pathology. Chin Med J (Engl) 2004; 117: 247-51.

[17] Li Q, Verma A, Han PY, *et al.* Diabetic eNOS knockout mice develop accelerated retinopathy. Invest Ophthalmol Vis Sci 2010; 51: 5240-6.

[18] Bek T. Epidemiology of Diabetic Retinopathy. 2nd ed. van Bijsterveld OP, Ed. London: Martin Dunitz Ltd 2000.

[19] Mizuno K, Koide T, Yoshimura M, Araie M. Neuroprotective effect and intraocular penetration of nipradilol, a beta-blocker with nitric oxide donative action. Invest Ophthalmol Vis Sci 2001; 42: 688-94.

[20] Geroski DH, Edelhauser HF. Drug delivery for posterior segment eye disease. Invest Ophthalmol Vis Sci 2000; 41: 961-4.

[21] Geroski DH, Edelhauser HF. Transscleral drug delivery for posterior segment disease. Adv Drug Deliv Rev 2001; 52: 37-48.

Received: December 5, 2010 Revised: December 27, 2010 Accepted: January 16, 2011

Nutrition and Dietary Supplements

Dovepress

ORIGINAL RESEARCH

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

Phan Nguyen Thanh Binh[1]
Kuniyasu Soda[2]
Masanobu Kawakami[3]

[1]Department of Food and Nutrition, Japan Women's University, Tokyo, Japan; [2]Cardiovascular Research Institute, [3]Department of Internal Medicine, Saitama Medical Center, Jichi Medical University, Saitama, Japan

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

Correspondence: Kuniyasu Soda
1-847 Amanuma, Saitama City, Saitama 330-0834, Japan
Tel +81 48 647 2111
Fax +81 48 648 5166
Email soda@jichi.ac.jp

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.

References

1. Bamia C, Trichopoulos D, Ferrari P, et al. Dietary patterns and survival of older Europeans: The EPIC-Elderly Study (European Prospective Investigation into Cancer and Nutrition). *Public Health Nutr*. 2007; 10(6):590–598.
2. Iestra J, Knoops K, Kromhout D, de Groot L, Grobbee D, van Staveren W. Lifestyle, Mediterranean diet and survival in European post-myocardial infarction patients. *Eur J Cardiovasc Prev Rehabil*. 2006;13(6):894–900.
3. Lagiou P, Trichopoulos D, Sandin S, et al. Mediterranean dietary pattern and mortality among young women: A cohort study in Sweden. *Br J Nutr*. 2006;96(2):384–392.
4. Keys A, Menotti A, Karvonen MJ, et al. The diet and 15-year death rate in the seven countries study. *Am J Epidemiol*. 1986;124(6): 903–915.
5. Tunstall-Pedoe H, Kuulasmaa K, Mahonen M, Tolonen H, Ruokokoski E, Amouyel P. Contribution of trends in survival and coronary-event rates to changes in coronary heart disease mortality: 10-year results from 37 WHO MONICA project populations. Monitoring trends and determinants in cardiovascular disease. *Lancet*. 1999;353(9164): 1547–1557.
6. Masia R, Pena A, Marrugat J, et al. High prevalence of cardiovascular risk factors in Gerona, Spain, a province with low myocardial infarction incidence. REGICOR Investigators. *J Epidemiol Community Health*. 1998;52(11):707–715.
7. Lichtenstein AH, Appel LJ, Brands M, et al. Summary of American Heart Association Diet and Lifestyle Recommendations Revision 2006. *Arterioscler Thromb Vasc Biol*. 2006;26(10):2186–2191.
8. Nishimura K, Araki N, Ohnishi Y, Kozaki S. Effects of dietary polyamine deficiency on Trypanosoma gambiense infection in rats. *Exp Parasitol*. 2001;97(2):95–101.
9. Bardocz S, Duguid TJ, Brown DS, et al. The importance of dietary polyamines in cell regeneration and growth. *Br J Nutr*. 1995;73(6): 819–828.
10. Uda K, Tsujikawa T, Fujiyama Y, Bamba T. Rapid absorption of luminal polyamines in a rat small intestine ex vivo model. *J Gastroenterol Hepatol*. 2003;18(5):554–559.
11. Kobayashi M, Xu YJ, Samejima K, et al. Fate of orally administered 15 N-labeled polyamines in rats bearing solid tumors. *Biol Pharm Bull*. 2003;26(3):285–288.
12. Bardócz S, Grant G, Brown DS, Ralph A, Pusztai A. Polyamines in food – implications for growth and health. *J Nutr Biochem*. 1993; 4(2):66–71.
13. Cipolla BG, Havouis R, Moulinoux JP. 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(2):203–212.
14. Nishibori N, Fujihara S, Akatuki T. Amounts of polyamines in foods in Japan and intake by Japanese. *Food Chem*. 2007;100(2): 491–497.
15. Sacks FM, Lichtenstein A, van Horn L, Harris W, Kris-Etherton P, Winston M. Soy protein, isoflavones, and cardiovascular health: An American Heart Association Science Advisory for professionals from the Nutrition Committee. *Circulation*. 2006;113(7): 1034–1044.
16. Zhang M, Caragine T, Wang H, et al. Spermine inhibits proinflammatory cytokine synthesis in human mononuclear cells: A counter-regulatory mechanism that restrains the immune response. *J Exp Med*. 1997;185(10):1759–1768.
17. Soda K, Kano Y, Nakamura T, Kasono K, Kawakami M, Konishi F. Spermine, a natural polyamine, suppresses LFA-1 expression on human lymphocyte. *J Immunol*. 2005;175(1):237–245.
18. Esposito K, Marfella R, Ciotola M, et al. Effect of a Mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: A randomized trial. *JAMA*. 2004;292(12):1440–1446.
19. Chrysohoou C, Panagiotakos DB, Pitsavos C, Das UN, Stefanadis C. Adherence to the Mediterranean diet attenuates inflammation and coagulation process in healthy adults: The ATTICA Study. *J Am Coll Cardiol*. 2004;44(1):152–158.
20. Lopez-Garcia E, Schulze MB, Fung TT, et al. Major dietary patterns are related to plasma concentrations of markers of inflammation and endothelial dysfunction. *Am J Clin Nutr*. 2004;80(4):1029–1035.
21. Satsu H, Hyun JS, Shin HS, Shimizu M. Suppressive effect of an isoflavone fraction on tumor necrosis factor-alpha-induced interleukin-8 production in human intestinal epithelial Caco-2 cells. *J Nutr Sci Vitaminol (Tokyo)*. 2009;55(5):442–446.
22. Calder PC. n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. *Am J Clin Nutr*. 2006;83 Suppl 6:S1505–S1519.
23. Soda K, Dobashi Y, Kano Y, Tsujinaka S, Konishi F. Polyamine-rich food decreases age-associated pathology and mortality in aged mice. *Exp Gerontol*. 2009;44(11):727–732.
24. Soda K, Kano Y, Sakuragi M, Takao K, Lefor A, Konishi F. Long-term oral polyamine intake increases blood polyamine concentrations. *J Nutr Sci Vitaminol (Tokyo)*. 2009;55(4):361–366.
25. Kouris-Blazos A, Gnardellis C, Wahlqvist ML, Trichopoulos D, Lukito W, Trichopoulou A. Are the advantages of the Mediterranean diet transferable to other populations? A cohort study in Melbourne, Australia. *Br J Nutr*. 1999;82(1):57–61.
26. Haveman-Nies A, de Groot LC, van Staveren WA. Dietary quality, lifestyle factors and healthy ageing in Europe: The SENECA study. *Age Ageing*. 2003;32(4):427–434.
27. Estruch R, Martinez-Gonzalez MA, Corella D, et al. Effects of a Mediterranean-style diet on cardiovascular risk factors: A randomized trial. *Ann Intern Med*. 2006;145(1):1–11.
28. Schulze MB, Hoffmann K, Manson JE, et al. Dietary pattern, inflammation, and incidence of type 2 diabetes in women. *Am J Clin Nutr*. 2005;82(3):675–684.
29. Lee TH, Hoover RL, Williams JD, et al. Effect of dietary enrichment with eicosapentaenoic and docosahexaenoic acids on in vitro neutrophil and monocyte leukotriene generation and neutrophil function. *N Engl J Med*. 1985;312(19):1217–1224.

30. Mehta JL, Lopez LM, Lawson D, Wargovich TJ, Williams LL. Dietary supplementation with omega-3 polyunsaturated fatty acids in patients with stable coronary heart disease. Effects on indices of platelet and neutrophil function and exercise performance. *Am J Med.* 1988;84(1):45–52.
31. Lefer AM, Lefer DJ. The role of nitric oxide and cell adhesion molecules on the microcirculation in ischaemia-reperfusion. *Cardiovasc Res.* 1996;32(4):743–751.
32. Drexler H, Zeiher AM, Meinzer K, Just H. Correction of endothelial dysfunction in coronary microcirculation of hypercholesterolaemic patients by L-arginine. *Lancet.* 1991;338(8782–3):1546–1550.
33. Cooke JP, Singer AH, Tsao P, Zera P, Rowan RA, Billingham ME. Antiatherogenic effects of L-arginine in the hypercholesterolemic rabbit. *J Clin Invest.* 1992;90(3):1168–1172.

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

ファン・グュエン・タン・ビン*, ** Phan Nguyen Thanh BINH
川上正舒*** Masanobu KAWAKAMI
阿部泰宣**** Yasunori ABE
為本浩至**** Hiroyuki TAMEMOTO
ファム・ギア・ティエン** Pham Gia TIEN
リ・グュエン・トルンドク・ソン** Le Nguyen Trung Duc SON
トラン・ティ・ミン・ハン** Tran Thi Minh HANH
ド・ティ・ノック・ディプ** Do Thi Ngoc DIEP
リ・ティ・キム・クイ** Le Thi Kim QUI
グュエン・ヴァン・チュエン* Nguyen Van CHUYEN
丸山千寿子* Chizuko MARUYAMA

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

* 日本女子大学人間生活学研究科人間発達学専攻, Division of Human Development, Graduate School of Human Life Science, Japan Women's University
** ホーチミン栄養センター HCMC Nutrition Center, HCMC, Vietnam
*** 自治医科大学附属さいたま医療センター Department of Internal Medicine, Jichi Medical University Saitama Medical Center,
**** 自治医科大学さいたま医療センター循環器病臨床医学研究所 Cardiovascular Research Institute, Saitama Medical Center, Jichi Medical University
***** 自治医科大学生化学講座 Department of Biochemistry, Jichi Medical University

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 $\geq$ 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 ≥ 7.0 mmol/l (126 mg/dl), 2-h ≥ 11.1 mmol/l (200 mg/dl); for IGT, fasting < 7.0 mmol/l (126 mg/dl) and 2-h ≥ 7.8 mmol/l (140 mg/dl) and < 11.1 mmol/l (200 mg/dl); and for IFG ≥ 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