の中で、症例１，２はＦＨヘテロ接合体のからの生体肝移植によって、HMG-CoA 還元酵素阻害薬等の薬物療法の補助下で良好な脂質コントロールが得られている。移植手術は大きな侵襲を伴うが、２症例とも大きな問題なく成長している。またドナーである両親も術後問題なく経過している。以上から、わずか２例ではあるが、日本人のＦＨホモ接合体においても、肝臓移植は非常に有効な治療法であると考えられる。

LDL 受容体変異のコンパウンドヘテロである症例３は、予想以上にスタチンの効果が認められ、約 45%の LDL-C 低下が得られている。早期にＬＤＬアフェレシスの導入が望ましいことは明らかだが、遺伝子変異の部位によって良好な薬物治療効果が期待できることは、ＬＤＬアフェレシス導入に問題のある症例において薬物治療を考慮する判断材料になると考えられる。

Ｅ．結論

ＦＨホモ接合体は代表的な難治性の原発性高脂血症であるが、我が国でもＬＤＬアフェレシスの普及によって生命予後が改善している。しかし希少疾患であるため国内からの報告は少数であり、ＬＤＬアフェレシス以外の治療法の有効性や問題点について、全国から症例を集積し検討していく必要がある。

Ｆ．健康危険情報

なし

Ｇ．研究発表

１．論文発表

1. Saito T, Hasegawa Y, **Ishigaki Y**, Yamada T, Gao J, Imai J, Uno K, Kaneko K, Ogihara T, Shimosawa T, Asano T, Fujita T, Oka Y, Katagiri H. Importance of endothelial NF-κB signalling in vascular remodelling and aortic aneurysm formation. Cardiovasc Res. 2013; 97(1):106-14.
2. Takahashi K, Yamada T, Tsukita S, Kaneko K, Shirai Y, Munakata Y, **Ishigaki Y**, Imai J, Uno K, Hasegawa Y, Sawada S, Oka Y, Katagiri H. Chronic mild stress alters circadian expressions of molecular clock genes in the liver. Am J Physiol Endocrinol Metab. 2013 ;304(3):E301-9.
3. Takagi M, **Ishigaki Y**, Uno K, Sawada S, Imai J, Kaneko K, Hasegawa Y, Yamada T, Tokita A, Iseki K, Kanno S, Nishio Y, Katagiri H, Mori E. Cognitive dysfunction associated with anti-glutamic acid decarboxylase autoimmunity: a case-control study. BMC Neurol. 2013;13:76.
4. Kondo K, **Ishigaki Y**, Gao J, Yamada T, Imai J, Sawada S, Muto A, Oka Y, Igarashi K, Katagiri H. Bach1 deficiency protects pancreatic β-cells from oxidative stress injury. Am J Physiol Endocrinol Metab. 2013; 305(5):E641-8.
5. Sakata N, Goto M, Motoi F, Hayashi H, Nakagawa K, Mizuma M, Yamaya H, Hasegawa Y, Yamaguchi S, Sawada S, Ottomo S, Okada T, Fukase K, Yoshida H, Ito T, Hirota

M, **Ishigaki Y**, Sekiguchi S, Rikiyama T, Katayose Y, Fujimori K, Egawa S, Shimosegawa T, Katagiri H, Satomi S, Unno M. Clinical Experiences in the Treatment of Pancreatic Arteriovenous Malformation by Total Pancreatectomy With Islet Autotransplantation. Transplantation. 2013; 96(5):e38-e40.

2．学会発表

1. 東海林　渉、**石垣　泰**、黒澤　聡子、山田　哲也、今井　淳太、澤田正二郎、鴇田　藍、宇野　健司、長谷川　豊、金子　慶三、片桐　秀樹、安保　英勇．糖尿病の食事療法への夫婦の取り組みタイプに関する検討：血糖コントロールおよびメンタルヘルスの比較．第 56 回日本糖尿病学会年次学術集会；2013；熊本
2. 児玉慎二郎、山田　哲也、江見　充、工藤　宏仁、石井　美穂、佐藤　秀則、澤田正二郎、今井　淳太、**石垣　泰**、岡　芳知、片桐　秀樹．全ゲノム CNV 解析による若年発症 2 型糖尿病患者における複数領域の高頻度ゲノム欠失．第 56 回日本糖尿病学会年次学術集会；2013；熊本
3. 澤田正二郎、**石垣　泰**、今井　淳太、山田　哲也、内藤　剛、柴田近、佐々木　巌、岡　芳知、片桐秀樹．糖尿病合併高度肥満に対する肥満手術1年後の持続血糖モニター（continuous glucose monitoring；CGM）で捉えた血糖プロフィール．第31回日本肥満症治療学会学術集会；2013；東京
4. 澤田正二郎、**石垣　泰**、鴇田　藍、長谷川英之、本蔵理恵子、金子　礼、今井　淳太、山田　哲也、宮地　幸哉、金井　浩、岡　芳知、片桐　秀樹．1 型と 2 型糖尿病患者の頸動脈血管弾性特性の比較検討．第 45 回日本動脈硬化学会学術集会；2013；東京
5. 村椿　智彦、**石垣　泰**、森下　城、鹿野　理子、金澤　素、片桐　秀樹、福土　審．肥満患者と健常者における食物刺激に対する渇望反応の差異．第 34 回日本肥満学会；2013；東京

H．知的財産権の出願・登録状況

1. 特許取得　なし
2. 実用新案登録　なし
3. その他　なし

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
該当なし							

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Sato Y, Nagao M, Asai A, et al.	Association of glycated albumin with the presence of carotid plaque in patients with type 2 diabetes.	J Diabetes Invest	4(6)	634-639	2013
Asai A, Nagao M, Kawahara M, et al	Effect of impaired glucose tolerance on atherosclerotic lesion formation: an evaluation in selectively bred mice with different susceptibilities to glucose intolerance.	Atherosclerosis	231	421-426	2013
Nagao M, Asai A, Inaba W, et al.	Characterization of pancreatic islets in two selectively bred mouse lines with different susceptibilities to high-fat diet-induced glucose intolerance.	PLoS ONE	9	1-7	2014
Nishizaki Y, Shimada K, Tani S, Ogawa T, Ando J, Takahashi M, Yamamoto M, Shinozaki T, Miyauchi K, Nagao K, Hirayama A, Yoshimura M, Komuro I, Nagai R, Daida H.	Significance of imbalance in the ratio of serum n-3 to n-6 polyunsaturated fatty acids in patients with acute coronary syndrome.	Am J Cardiol	113(3)	441-445	2014
Teramoto T, Sasaki J, Ishibashi S, Birou S, Daida H, Dohi S, Egusa G, Hiro T, Hirobe K, Iida M, Kihara S, Kinoshita M, Maruyama C, Ohta T, Okamura T, Yamashita S, Yokode M, Yokote K; Japan Atherosclerosis Society.	Executive summary of the Japan Atherosclerosis Society (JAS) guidelines for the diagnosis and prevention of atherosclerotic cardiovascular diseases in Japan - 2012 version.	J Atheroscler Thromb	20(6)	517-523	2012

Dohi T, Miyauchi K, Ohkawa R, Nakamura K, Kurano M, Kishimoto T, Yanagisawa N, Ogita M, Miyazaki T, Nishino A, Yaginuma K, Tamura H, Kojima T, Yokoyama K, Kurata T, Shimada K, Daida H, Yatomi Y.	Increased lysophosphatidic acid levels in culprit coronary arteries of patients with acute coronary syndrome.	Atherosclerosis	229	192-197	2013
Sai E, Shimada K, Yokoyama T, Sato S, Miyazaki T, Hiki M, Tamura Y, Aoki S, Watada H, Kawamori R, Daida H.	Association between myocardial triglyceride content and cardiac function in healthy subjects and endurance athletes.	PLoS One	8(4)	1-6	2013
Ogita M, Miyauchi K, Miyazaki T, Naito R, Konishi H, Tsuboi S, Dohi T, Kasai T, Yokoyama T, Okazaki S, Kurata T, Daida H.	Low high-density lipoprotein cholesterol is a residual risk factor associated with long-term clinical outcomes in diabetic patients with stable coronary artery disease who achieve optimal control of low-density lipoprotein cholesterol	Heart Vessels	10	35-41	2014
Daida H, Iwase T, Yagi S, Ando H, Nakajima H.	Effect of darapladib on plasma lipoprotein-associated phospholipase A2 activity in Japanese dyslipidemic patients, with exploratory analysis of a PLA2G7 gene polymorphism of Val279	Circ J	77	1518-1525	2013
Matsumori R, Miyazaki T, Shimada K, Kume A, Kitamura Y, Oshida K, Yanagisawa N, Kiyanagi T, Hiki M, Fukao K, Hirose K, Ohsaka H, Mokuno H, Daida H.	High levels of very long-chain saturated fatty acid in erythrocytes correlates with atherogenic lipoprotein profiles in subjects with metabolic syndrome.	Diabetes Res ClinPract	99	12-18	2013
Kasai T, Miyauchi K, Yanagisawa N, Kajimoto K, Kubota N, Ogita M, Tsuboi S, Amano A, Daida H.	Mortality risk of triglyceride levels in patients with coronary artery disease.	Heart	99	22-29	2013
Naito S, Kamata M, Furuya M, Hayashi M, Kuroda M, Bujo H, Kamata K.	Amelioration of circulating lipoprotein profile and proteinuria in a patient with LCAT deficiency due to a novel mutation (Cys74Tyr) in the lid region of LCAT under a fat-restricted diet and ARB treatment.	Atherosclerosis	228	193-197	2013

Arai H, Kokubo Y, Watanabe M, Sawamura T, Ito Y, Minagawa A, Okamura T, Miyamato Y.	Small Dense Low-Density Lipoproteins Cholesterol can Predict Incident Cardiovascular Disease in an Urban Japanese Cohort: The Suita Study.	J Atheroscler Thromb	20(2)	195-203	2013
Nozue T, Hattori H, Ishihara M, Iwasaki T, Hirano T, Kawashiri MA, Yamagishi M, Michishita I.	Comparison of effects of pitavastatin versus pravastatin on serum proprotein convertase subtilisin/kexin type 9 levels in statin-naive patients with coronary artery disease.	Am J Cardiol	111(10)	1415-1419	2013
Yamaaki N, Yagi K, Kobayashi J, Nohara A, Ito N, Asano A, Nakano K, Liu J, Okamoto T, Mori Y, Ohbatake A, Okazaki S, Takeda Y, Yamagishi M.	Impact of serum retinol-binding protein 4 levels on regulation of remnant-like particles triglyceride in type 2 diabetes mellitus.	J Diabetes Res.		1-5	2013
Fujimoto Y, Nakagawa Y, Satoh A, Okuda K, Shingyouchi A, Naka A, Matsuzaka T, Iwasaki H, Kobayashi K, Yahagi N, Shimada M, Yatoh S, Suzuki H, Yogosawa S, Izumi T, Sone H, Urayama O, Yamada N, Shimano H.	TFE3 Controls Lipid Metabolism in Adipose Tissue of Male Mice by Suppressing Lipolysis and Thermogenesis.	Endocrinology	154(10)	3577-3588	2013
Ishida T, Ohta M, Nakakuki M, Kami H, Uchiyama R, Kawano H, Notsu T, Imada K, Shimano H.	Distinct regulation of plasma LDL cholesterol by eicosapentaenoic acid and docosahexaenoic acid in high fat diet-fed hamsters: Participation of cholesterol ester transfer protein and LDL receptor.	Prostaglandins Leukot Essent Fatty Acids	88	281-288	2013
Fujihara K, Suzuki H, Sato A, Kodama S, Heianza Y, Saito K, Iwasaki H, Kobayashi K, Yatoh S, Takahashi A, Yamada N, Sone H, Shimano H.	Carotid Artery Plaque and LDL-to-HDL Cholesterol Ratio Predict Atherosclerotic Status in Coronary Arteries in Asymptomatic Patients with Type 2 Diabetes Mellitus.	J Atheroscler Thromb	20(5)	452-464	2012
Hayashi T, Araki A, Kawashima S, Sone H, Watanabe H, Ohrui T, Yokote K, Takemoto M, Kubota K, Noda M, Noto H, Ina K, Nomura H; Japan CDM group.	Metabolic predictors of ischemic heart disease and cerebrovascular attack in elderly diabetic individuals: difference in risk by age.	Cardiovasc Diabetol.	12	1-11	2013

Sun L, Ishida T, Miyashita K, Kinoshita N, Mori K, Yasuda T, Toh R, Nakajima K, Imamura S, Hirata K.	Plasma activity of endothelial lipase impacts high-density lipoprotein metabolism and coronary risk factors in humans.	J Atheroscler Thromb	20	1-9	2013
Sasaki-Miyamoto M, Yasuda T, Monguchi T, Nakajima H, Mori K, Toh R, Ishida T, Hirata K.	Pitavastatin increases HDL particles functionally- preserved with cholesterol efflux capacity and anti-oxidative action in dyslipidemia patients.	J Atheroscler Thromb	20(9)	708-716	2013
Yamamoto T, Izumi – Yamamoto K, Iizuka Y, Shirota M, Nagase M, Fujita T, & Gotoda T.	A novel link between Slc22a18 and fat accumulation revealed by a mutation in the spontaneously hypertensive rat.	Biochem Biophys Res Commun	440	521-526	2013
Takase S, Osuga JI, Fujita H, Hara K, Sekiya M, Igarashi M, Takanashi M, Takeuchi Y, Izumida Y, Ohta K, Kumagai M, Nishi M, Kubota M, Masuda Y, Taira Y, Okazaki S, Iizuka Y, Yahagi N, Ohashi K, Yoshida H, Yanai H, Tada N, Gotoda T, Ishibashi S, Kadowaki T, & Okazaki H.	Apolipoprotein C-ll deficiency with no rare variant in the APOC2 gene.	J Atheroscler Thromb	20(5)	481-493	2013
Yamamoto T, Obika S, Nakatani M, Yasuhara H, Wada F, Shibata E, Shibata MA, Harada-Shiba M.	Locked nucleic acid antisense inhibitor targeting apolipoprotein C-III efficiently and preferentially removed triglyceride from large VLDL particles from murine plasma.	European Journal of Pharmacology	723	353-359	2014
Teramoto T, Sasaki J, Harada-Shiba M, Arai H, Ishibasi S, Birou S, Bujo H, Daida H, Dohi S, Egusa G, Hiro T, Hirobe K, Iida M, Kihara S, Kinoshita M, Maruyama C, Nohara A, Ohta T, Oikawa S, Okada T, Okamura T, Watatsuki A, Yamashita S, Yokode M, Yokote K.	Familial hypercholesterolemia, Executive summary of the Japan Atherosclerosis Society(JAS) Guidelines for diagnosis and prevention of atherosclerotic cardiovascular diseases in Japan-2012 version.	J Atheroscler Thromb	21(1)	6-10	2013
Kobayashi K, Nagata E, Sasaki K, Harada-Shiba M, Kojo S, Kikuzaki H.	Increase in Secretory Sphingomyelinase Activity and Specific Ceramides in the Aorta of Apolipoprotein E Knockout Mice during Aging.	Biol. Pharm. Bull	36(7)	1192-1196	2013

Terasaki F, Morita H, Harada-Shiba M, Ohta N, Otsuka K, Nogi S, Miyamura M, Suzuki S, Ito T, Shimomura H, Katsumata T, Miyamoto Y, Ishizaka N.	Familial Hypercholesterolemia with Multiple Large Tendinous Xanthomas and Advanced coronary Artery Atherosclerosis.	Intern Med.	52	577-581	2013
Saito T, Hasegawa Y, Ishigaki Y, Yamada T, Gao J, Imai J, Uno K, Kaneko K, Ogihara T, Shimosawa T, Asano T, Fujita T, Oka Y, Katagiri H.	Importance of endothelial NF-κB signalling in vascular remodelling and aortic aneurysm formation.	Cardiovasc Res	97	106-114	2013
Takahashi K, Yamada T, Tsukita S, Kaneko K, Shirai Y, Munakata Y, Ishigaki Y, Imai J, Uno K, Hasegawa Y, Sawada S, Oka Y, Katagiri H.	Chronic mild stress alters circadian expressions of molecular clock genes in the liver.	Am J Physiol Endocrinol Metab	304	E301-309	2013
Kondo K, Ishigaki Y, Gao J, Yamada T, Imai J, Sawada S, Muto A, Oka Y, Igarashi K, Katagiri H.	Bach1 deficiency protects pancreatic β-cells from oxidative stress injury.	Am J Physiol Endocrinol Metab	305	E641-648	2013

Association of glycated albumin with the presence of carotid plaque in patients with type 2 diabetes

Yuki Sato, Mototsugu Nagao, Akira Asai, Yasushi Nakajima, Makiyo Takaya, Naomi Takeichi, Shuji Takemitsu, Mariko Sudo, Toshiko Kano-Wakakuri, Akira Ishizaki, Taro Harada, Kyoko Tanimura-Inagaki, Fumitaka Okajima, Hideki Tamura, Hitoshi Sugihara, Shinichi Oikawa*

ABSTRACT

Aims/Introduction: Postprandial hyperglycemia is a potent risk factor for cardiovascular disease. Serum glycated albumin (GA) has been reported to reflect postprandial blood glucose fluctuations. In the present study, we assessed the possible correlation of GA with the presence of carotid plaque to evaluate the potential clinical usefulness of GA for predicting atherosclerotic cardiovascular complications in patients with type 2 diabetes.

Materials and Methods: Patients with type 2 diabetes (n = 236) admitted to Nippon Medical School Hospital (Tokyo, Japan) for glycemic control (aged 19–86 years, 81 females and 155 males) were examined. Clinical measurements were taken on admission. The presence of carotid plaque was assessed by ultrasonography.

Results: In patients with carotid plaque (n = 154), GA (P = 0.023) was higher than those without carotid plaque (n = 82). In contrast, neither fasting plasma glucose (P = 0.48) nor glycated hemoglobin (P = 0.41) was significantly different between the groups. The results of logistic regression analysis showed that GA (age- and sex-adjusted odds ratio [95% confidence interval], 1.05 [1.01–1.09]; P = 0.017) and glycated hemoglobin (1.17 [1.01–1.37]; P = 0.036) were significantly associated with the presence of carotid plaque.

Conclusions: The positive correlation of serum GA with the presence of carotid plaque in type 2 diabetes suggests that GA will serve as a useful clinical marker for predicting diabetic cardiovascular complications. (J Diabetes Invest, doi: 10.1111/jdi.12085, 2013)

KEY WORDS: Carotid plaque, Glycated albumin, Type 2 diabetes mellitus

INTRODUCTION

Glycated hemoglobin (HbA_{1c}) is regarded as a gold standard for monitoring glycemic control. Most expert committees now recommend the use of HbA_{1c} in the diagnosis of diabetes[1]. Although the overall usefulness of HbA_{1c} is well accepted, epidemiological data suggest that HbA_{1c} is not always versatile for predicting all types of diabetic complication; namely, whereas HbA_{1c} is a good predictor of microvascular complications, it appears to be less so for macrovascular outcomes[2]. One interpretation is that the fluctuation of blood glucose contributes more to macrovascular diabetic complications than time-averaged blood glucose concentration represented by HbA_{1c}[2]. Postprandial acute glucose fluctuations are postulated to contribute to the pathogenesis of atherosclerotic cardiovascular complications through the induction of oxidative stress and consequent endothelial dysfunction[3–5].

Division of Endocrinology and Metabolism, Department of Medicine, Nippon Medical School, Tokyo, Japan
*Corresponding author. Shinichi Oikawa Tel.: +81 3 3822 2131 Fax: +81 3 5802 8153
E-mail address: shinichi@nms.ac.jp
Received 29 November 2012; revised 29 January 2013; accepted 1 March 2013

Whereas glycation of hemoglobin in erythrocytes (i.e., HbA_{1c}) might not reflect blood glucose fluctuations, several reports have shown that glycation of serum albumin reflects the glycemic excursions[6,7]. The glycation of serum proteins has been long assessed by the measurement of fructosamine; however, the reduction reaction-based colorimetric assay is influenced by protein concentration and other coexisting substances in serum[8]. For assessing serum protein glycation more accurately, glycated albumin (GA) assay has been developed. GA was originally analyzed by high-performance liquid chromatography (HPLC), but it can currently be determined with automated clinical analyzers by a rapid and specific enzymatic method[9]. The measurement of GA is now available for monitoring glycemic control in patients with diabetes under public health insurance coverage in Japan. GA can be a potential index for predicting cardiovascular events, as it reflects blood glucose fluctuations better than HbA_{1c}[6,7]. Postprandial hyperglycemia is epidemiologically a more potent risk factor for diabetic cardiovascular complications than fasting plasma glucose (FPG)[10,11].

In the present study, we assessed the association of GA with the presence of carotid plaque, a surrogate marker for atherosclerotic disease, to evaluate the potential usefulness of the measurement of GA for predicting cardiovascular complications in type 2 diabetes.

MATERIALS AND METHODS

Participants

Patients with type 2 diabetes who were admitted to Nippon Medical School Hospital (Tokyo, Japan) for glycemic control during 2005–2012 were enrolled in the present study (n = 236, aged 19–86 years, 81 females and 155 males). Exclusion criteria included diabetic nephropathy stage 3 or higher (urinary albumin exertion ≥300 mg/g·Cr [spot], or ≥300 mg/day; Joint Committee of the Japan Diabetes Society and the Japanese Society of Nephrology[12]), uncontrolled endocrine disease, steroid treatment, ketoacidosis, estimated primary hyperlipidemia, malignant disease, anemia (hemoglobin <10.0 g/dL) and other systemic disorders. The present study was approved by the Institutional Review Board, and all participants were enrolled after giving informed consent.

Clinical Measurements

All participants underwent a physical examination including height, bodyweight and blood pressure on the first morning of admission. Blood sample was taken after an overnight fast. FPG, HbA_{1c} and serum GA were measured by the glucose oxidase method (ADAMS Glucose GA-1170; Arkray, Kyoto, Japan), HPLC (ADAMS A1c HA-8160; Arkray) and an enzymatic method using albumin-specific proteinase and ketoamine oxidase (Lucica GA-L; Asahi Kasei Pharma, Tokyo, Japan), respectively. HbA_{1c} level was expressed as the percentage value of the National Glycohemoglobin Standardization Program according to the guideline of the Japan Diabetes Society[13,14]. GA was expressed as a percentage of total serum albumin. Serum total cholesterol, high-density lipoprotein (HDL)-cholesterol and triacylglycerol were measured enzymatically (Sekisui Medical, Tokyo, Japan). Low-density lipoprotein cholesterol concentration was calculated by the Friedewald formula[15]. Smoking habit (current, past or never) and type 2 diabetes duration were assessed by interview.

Carotid Ultrasonography

Carotid artery status was examined with high-resolution B-mode ultrasound systems (SDU-2000; Shimadzu, Kyoto, Japan; iU22 and EnVisor; Philips Healthcare, Andover, MA, USA; LOGIQ 7; GE Healthcare, Little Chalfont, England, UK) equipped with linear transducers with a frequency of 3–12 MHz as previously described[16]. Carotid plaque was defined as a focal intima-media thickening ≥1.0 mm with marked protrusion into the lumen.

Statistical Analysis

Continuous variables are expressed as means ± standard deviation or median (interquartile range) for variables with normal or skewed distribution, respectively. Differences in clinical data between participants with and without carotid plaque were assessed by Student's t-test, Mann–Whitney U-test or χ^2-test as appropriate. Correlations between GA and other continuous variables were examined by Pearson's correlation analysis. A logistic regression model was applied to determine the odds ratio for the presence of carotid plaque. A P-value of <0.05 was considered as significant. All analyses were carried out with JMP software (version 9.0; SAS Institute, Cary, NC, USA).

RESULTS

Of 236 participants enrolled in the present study, 154 (65%) had carotid plaque. The clinical characteristics for each group are shown in Table 1. In the participants with carotid plaque, age was higher and the duration of type 2 diabetes was longer than in those without carotid plaque. Body mass index (BMI) was lower in the participants with carotid plaque compared with those without. In glycemic control indices, GA and GA-to-HbA_{1c} ratio (GA/HbA_{1c}) were higher in the participants with carotid plaque than in those without, whereas neither FPG nor HbA_{1c} was significantly different between the two groups. With regard to diabetic complications, the participants with carotid plaque had a higher incidence of retinopathy or abnormal Achilles tendon reflex than those without. Regarding prehospital medication, a higher population of the participants with carotid plaque had been treated with an antihypertensive agent or antiplatelet agent than those without.

Table 2 shows Pearson's correlation coefficients between GA and other continuous variables. GA was positively correlated with FPG, HbA_{1c} and HDL-cholesterol, whereas inversely with systolic blood pressure, diastolic blood pressure and BMI.

Table 3 shows the results of logistic regression analysis for the presence of carotid plaque. Age, duration of type 2 diabetes, GA and GA/HbA_{1c} were positively associated with the presence of carotid plaque in unadjusted univariate analysis, whereas BMI was inversely associated with that. After the analysis was adjusted for age and sex, GA and HbA_{1c} were significant predictors of the carotid plaque presence. In contrast, no significant association was found between GA/HbA_{1c} and the presence of carotid plaque after the adjustment. To address the discrepancies between these glycemic control indices (GA, HbA_{1c} and GA/HbA_{1c}) and the carotid plaque presence with or without the adjustment, we analyzed the correlations between these glycemic control indices and age. Whereas no correlation was found between GA and age (Figure 1a), HbA1c was inversely correlated with age (Figure 1b). As a consequence, GA/HbA1c was positively correlated with age (Figure 1c).

DISCUSSION

HbA_{1c} and GA basically provide similar clinical information on recent glycemic control. However, owing to the shorter half-life of serum albumin than erythrocyte hemoglobin, GA reflects shorter-term blood glucose concentration (over 2–3 weeks) compared with HbA_{1c}, which reflects that over 2–3 months[17].

Table 1 | Clinical characteristics of the participants

Variable	All participants	Carotid plaque		*P*-value*
	(*n* = 236)	+ (*n* = 154)	– (*n* = 82)	
Age (years)	56 ± 13	60 ± 11	50 ± 14	<0.0001
Sex (female/male)	81/155	52/102	29/53	0.81
Duration of type 2 diabetes (years)	5 [0–11]	6 [1–15]	3 [0–10]	0.0069
Systolic blood pressure (mmHg)	127 ± 15	128 ± 15	125 ± 15	0.073
Diastolic blood pressure (mmHg)	75 ± 11	75 ± 10	74 ± 11	0.58
BMI (kg/m^2)	25.4 ± 4.9	24.8 ± 4.5	26.4 ± 5.6	0.014
Smoking habit, current or past (*n* [%])	148 [63]	102 [66]	46 [56]	0.13
Fasting plasma glucose (mmol/L)	9.79 ± 2.99	9.89 ± 3.04	9.60 ± 2.89	0.48
HbA_{1c} (%)	10.3 ± 2.1	10.4 ± 2.1	10.1 ± 1.9	0.41
GA (%)	28.5 ± 8.4	29.4 ± 8.9	26.8 ± 7.0	0.023
GA/HbA_{1c}	2.74 ± 0.45	2.80 ± 0.45	2.63 ± 0.43	0.0037
Total cholesterol (mmol/L)	5.31 ± 1.06	5.28 ± 1.06	5.37 ± 1.07	0.55
HDL-cholesterol (mmol/L)	1.27 ± 0.35	1.26 ± 0.32	1.27 ± 0.41	0.89
LDL-cholesterol (mmol/L)†	3.26 ± 0.89	3.26 ± 0.89	3.25 ± 0.88	0.93
Non HDL-cholesterol (mmol/L)	4.05 ± 1.05	4.02 ± 1.04	4.10 ± 1.08	0.58
Triacylglycerols (mmol/L)	1.72 ± 0.94	1.68 ± 0.88	1.81 ± 1.05	0.31
Retinopathy (*n* [%])	49 [21]	41 [27]	8 [10]	0.0014
Albuminuria, >30 mg/mg·Cr (*n* [%])	36 [15]	26 [17]	10 [12]	0.33
Abnormal Achilles tendon reflex (*n* [%])	98 [42]	75 [49]	23 [28]	0.0019
Prehospital medication				
Insulin (*n* [%])	29 [12]	23 [15]	6 [7]	0.078
Oral hypoglycemic agent (*n* [%])	120 [51]	81 [53]	39 [48]	0.46
Statin (*n* [%])	47 [20]	36 [23]	11 [13]	0.061
Antihypertensive agent (*n* [%])	75 [32]	57 [37]	18 [22]	0.016
Antiplatelet agent (*n* [%])	26 [11]	23 [15]	3 [4]	0.0043

Continuous variables are expressed as means ± SD or median [interquartile range]. *For differences between the subjects with (+) and without (–) carotid plaque. †As low-density lipoprotein (LDL)-cholesterol was calculated by the Friedewald formula, three participants (one in + and two in –) with hypertriacylglycerolemia (≥4.5 mmol/L) were excluded from the statistical analysis of LDL-cholesterol. BMI, body mass index; GA, glycated albumin; GA/HbA_{1c}, glycated albumin-to-glycated hemoglobin ratio; HbA_{1c}, glycated hemoglobin; HDL, high-density lipoprotein.

GA should therefore be a more suitable index of glycemic control than HbA_{1c} in cases where rapid changes might occur in blood glucose concentration; for example, when starting or changing diabetes treatments[6,9,18]. GA is also proposed as a useful measure of glycemic control in populations for whom HbA_{1c} might not reflect glycemic status accurately; that is, anemics, those with hemoglobinopathies or neonates[19]. More intriguingly, GA has been reported to reflect blood glucose fluctuations[7,20]. A recent report clearly showed that GA, but not HbA_{1c}, was positively associated with glycemic excursions, which were assessed with 48-h continuous glucose monitoring[20].

Because GA has been reported to reflect blood glucose fluctuations and individuals with greater postprandial glycemic excursions are more likely to develop cardiovascular disease[10,11], GA might serve as a useful marker for predicting diabetic cardiovascular complications. A recent study actually reported that GA, but not HbA_{1c}, was associated with the increasing degree of coronary stenosis in type 2 diabetes[21]. The higher GA level in subjects with carotid plaque among the present participants (Table 1) also supports the clinical usefulness of GA for evaluating the risk of cardiovascular complications.

The positive correlations of GA with other glycemic indices (FPG and HbA_{1c}) suggest that GA increases with the deterioration of overall glycemic control (Table 2). Furthermore, GA was associated with the presence of carotid plaque, and the association remained significant after the logistic regression model was adjusted for age and sex (Table 3). As a recent meta-analysis of prospective cohorts[22] and an additional relevant report[23] showed, there is no doubt that higher HbA_{1c} level is associated with the risk of cardiovascular events in the general population without diabetes. However, in subjects with type 2 diabetes, epidemiological evidence indicates a weaker association of HbA_{1c} with macrovascular outcomes than that with microvascular complications[24]. Ishizaka *et al.*[25] actually demonstrated that HbA_{1c} level was strongly associated with the presence of carotid plaque in normal subjects (normal FPG and normal glucose tolerance); however, the association was not observed in subjects with diabetes or prediabetes. Similar to that report, no significant difference was found in HbA_{1c} between the subjects with

Table 2 | Pearson's correlation coefficients between glycated albumin and other continuous variables

Variable	*r*	*P*-value
Age	0.0077	0.91
Duration of type 2 diabetes	−0.041	0.53
Systolic blood pressure	−0.19	0.0028
Diastolic blood pressure	−0.13	0.046
BMI	−0.39	<0.0001
Fasting plasma glucose	0.71	<0.0001
HbA_{1c}	0.83	<0.0001
Total cholesterol	0.11	0.10
HDL-cholesterol	0.15	0.022
LDL-cholesterol*	0.083	0.20
Non HDL-cholesterol	0.056	0.39
Triacylglycerols	−0.044	0.50

*Three participants were excluded from the statistical analysis of low-density lipoprotein (LDL)-cholesterol (see the footnote to Table 1). BMI, body mass index; HbA_{1c}, glycated hemoglobin; HDL, high-density lipoprotein.

and without carotid plaque in the present study (Table 1). As patients with poor glycemic control were enrolled in the present study, their higher HbA_{1c} levels within a narrow range might be one reason for the lack of association. However, age- and sex-adjusted logistic regression analysis showed the association between HbA_{1c} and carotid plaque prevalence (Table 3).

Even though we excluded patients with anemia (hemoglobin <10.0 g/dL) and renal failure (stage 3 or higher), HbA_{1c} showed a significant inverse correlation with age in the present participants (Figure 1b). Several reports suggested that HbA_{1c} can be affected by age[26,27] and ethnic group[28,29]. Recently, Khoo *et al.*[30] reported a discrepancy between the oral glucose tolerance test (OGTT) and HbA_{1c} to diagnose diabetes in elderly populations. They suggested a higher false negative rate when diagnosing diabetes by HbA_{1c} alone as compared with by OGTT. These results suggest that HbA_{1c} alone is not enough to evaluate glycemic control, especially in elderly patients with type 2 diabetes. Conversely, as GA was not affected by age (Figure 1a), the measurement of GA might have clinical value for evaluating glycemic control more accurately, and for predicting atherosclerotic cardiovascular outcomes, especially in patients with type 2 diabetes.

As GA reflects blood glucose fluctuations better than HbA_{1c}[6,7], GA/HbA_{1c} might reflect the postprandial glycemic response and could be useful for predicting diabetic complications[31]. Most recently, possible clinical use of both GA and GA/HbA_{1c} for predicting the presence of carotid atherosclerosis[32] and the progression of intima-media thickness[33] were also reported in outpatients with type 2 diabetes. However, in the present study, the association between GA/HbA_{1c} and the presence of carotid atherosclerosis was not significant after the adjustment for age and sex. Similarly, no significant correlation was found between GA/HbA_{1c} and intima-media thickness after the adjustment (data not shown). One of the possible reasons for the differences from the previous reports[32,33] is the age distribution. Relative to the participants in those reports (aged 40–70 years and 53–68 years in the reports of Moon *et al.*[32] and Song *et al.*[33], respectively), the present participants had a wider age distribution (19–86 years). As HbA_{1c} was inversely correlated with age (Figure 1b), GA/HbA_{1c} (the index defined by reciprocal

Table 3 | Odds ratios of variables for the presence of plaque

Variable	Unadjusted		Age- and sex-adjusted	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Age (per 1 year)	1.07 (1.04–1.09)	<0.0001		
Sex (male)*	1.07 (0.61–1.88)	0.81		
Duration of type 2 diabetes (per 1 year)	1.05 (1.01–1.09)	0.0050	1.01 (0.98–1.05)	0.51
BMI (per 1 kg/m^2)	0.93 (0.88–0.99)	0.015	0.98 (0.92–1.04)	0.49
Smoking habit (current or past)†	1.54 (0.89–2.66)	0.13	1.80 (0.97–3.37)	0.062
Fasting plasma glucose (per 1 mmol/L)	1.03 (0.94–1.13)	0.48	1.06 (0.96–1.17)	0.24
HbA_{1c} (per 1%)	1.06 (0.93–1.21)	0.41	1.17 (1.01–1.37)	0.036
GA (per 1%)	1.04 (1.01–1.08)	0.020	1.05 (1.01–1.09)	0.017
GA/HbA_{1c} (per 1)	2.57 (1.36–5.05)	0.0032	1.74 (0.87–3.60)	0.12
Total cholesterol (per 1 mmol/L)	0.93 (0.72–1.19)	0.55	1.00 (0.76–1.32)	0.98
HDL-cholesterol (per 1 mmol/L)	0.95 (0.45–2.05)	0.89	0.42 (0.17–1.01)	0.052
Non HDL-cholesterol (per 1 mmol/L)	0.93 (0.72–1.20)	0.58	1.09 (0.82–1.44)	0.56
LDL-cholesterol (per 1 mmol/L)‡	1.01 (0.75–1.38)	0.93	1.08 (0.78–1.52)	0.63
Triacylglycerols (per 1 mmol/L)	0.87 (0.65–1.15)	0.31	1.15 (0.85–1.59)	0.37

BMI, body mass index; CI, confidence interval; GA, glycated albumin; GA/HbA_{1c}, glycated albumin-to-glycated hemoglobin ratio; HbA_{1c}, glycated hemoglobin; HDL, high-density lipoprotein; OR, odds ratio. *Female as reference. †Never as reference. ‡Three participants were excluded from the statistical analysis of low-density lipoprotein (LDL)-cholesterol (see the footnote to Table 1).

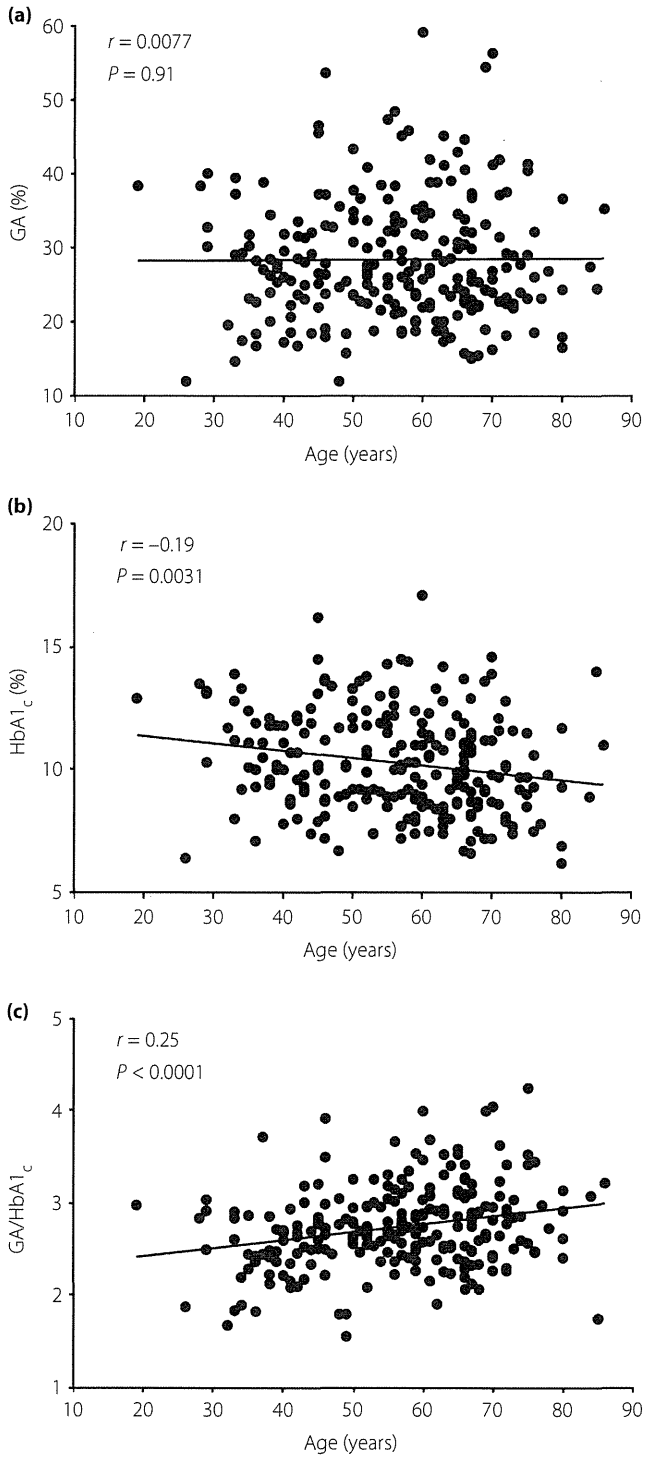

Figure 1 | Correlations of (a) glycated albumin (GA), (b) glycated hemoglobin (HbA_{1c}) and (c) GA-to-HbA_{1c} ratio (GA/HbA_{1c}) with age in the participants. Pearson's correlation coefficient is shown in each panel.

value of HbA_{1c}) showed a positive correlation with age in the present study (Figure 1c). These results suggest that age-related differences among the glycemic control indices should be taken into account for clinical use of GA/HbA_{1c} as a surrogate marker of diabetic complications.

In the present study, BMI was inversely correlated with GA (Table 2). Although the reasons remain unknown, several reports also showed that BMI was inversely correlated with GA[34–36]. Further investigation into the causes of the relationship between GA and BMI might provide new insights into the interpretation of glycemic control indices in the research and clinical practice of diabetes and its complications.

In conclusion, the present results showed that GA and GA/HbA_{1c} were higher in subjects with carotid plaque among patients with type 2 diabetes. Logistic regression analysis showed the positive association of GA and HbA_{1c} with the presence of carotid plaque when the models were adjusted for age and sex. Because this was a cross-sectional study, the present data provide just a snapshot of each patient at admission; this is a limitation of the present study. As atherosclerotic lesion formation is a longitudinal event, the longstanding history of vascular conditions should be taken into account. Nevertheless, the present cross-sectional data should warrant further prospective investigation to evaluate the clinical usefulness of GA for predicting cardiovascular outcomes in patients with type 2 diabetes.

ACKNOWLEDGEMENTS

The authors declare that there is no duality of interest associated with this manuscript.

REFERENCES

1. Kilpatrick ES, Bloomgarden ZT, Zimmet PZ. International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes: response to the International Expert Committee. *Diabetes Care* 2009; 32: 159–160.
2. Cohen RM, Smith EP. Frequency of HbA1c discordance in estimating blood glucose control. *Curr Opin Clin Nutr Metab Care* 2008; 11: 512–517.
3. Ceriello A, Esposito K, Piconi L, *et al.* Oscillating glucose is more deleterious to endothelial function and oxidative stress than mean glucose in normal and type 2 diabetic patients. *Diabetes* 2008; 57: 1349–1354.
4. Ceriello A. Postprandial hyperglycemia and diabetes complications: is it time to treat? *Diabetes* 2005; 54: 1–7.
5. Monnier L, Mas E, Ginet C, *et al.* Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. *JAMA* 2006; 295: 1681–1687.
6. Takahashi S, Uchino H, Shimizu T, *et al.* Comparison of glycated albumin (GA) and glycated hemoglobin (HbA1c) in type 2 diabetic patients: usefulness of GA for evaluation of short-term changes in glycemic control. *Endocr J* 2007; 54: 139–144.
7. Yoshiuchi K, Matsuhisa M, Katakami N, *et al.* Glycated albumin is a better indicator for glucose excursion than glycated hemoglobin in type 1 and type 2 diabetes. *Endocr J* 2008; 55: 503–507.

8. Armbruster DA. Fructosamine: structure, analysis, and clinical usefulness. *Clin Chem* 1987; 33: 2153–2163.
9. Kohzuma T, Koga M. Lucica GA-L glycated albumin assay kit: a new diagnostic test for diabetes mellitus. *Mol Diagn Ther* 2010; 14: 49–51.
10. Bonora E, Muggeo M. Postprandial blood glucose as a risk factor for cardiovascular disease in Type II diabetes: the epidemiological evidence. *Diabetologia* 2001; 44: 2107–2114.
11. Ceriello A, Hanefeld M, Leiter L, *et al.* Postprandial glucose regulation and diabetic complications. *Arch Intern Med* 2004; 164: 2090–2095.
12. Inomata S, Haneda M, Moriya T, *et al.* Revised criteria for the early diagnosis of diabetic nephropathy. *J Jpn Diabetes Soc* 2005; 48: 757–759 (Japanese).
13. Seino Y, Nanjo K, Tajima N, *et al.* Report of the Committee on the classification and diagnostic criteria of diabetes mellitus. *J Diabetes Invest* 2010; 1: 212–228.
14. Kashiwagi A, Kasuga M, Araki E, *et al.* International clinical harmonization of glycated hemoglobin in Japan: from Japan Diabetes Society to National Glycohemoglobin Standardization Program values. *J Diabetes Invest* 2012; 3: 39–40.
15. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; 18: 499–502.
16. Tanimura K, Nakajima Y, Nagao M, *et al.* Association of serum apolipoprotein B48 level with the presence of carotid plaque in type 2 diabetes mellitus. *Diabetes Res Clin Pract* 2008; 81: 338–344.
17. Tahara Y, Shima K. Kinetics of HbA1c, glycated albumin, and fructosamine and analysis of their weight functions against preceding plasma glucose level. *Diabetes Care* 1995; 18: 440–447.
18. Koga M, Murai J, Saito H, *et al.* Prediction of near-future glycated hemoglobin levels using glycated albumin levels before and after treatment for diabetes. *J Diabetes Invest* 2011; 2: 304–309.
19. Koga M, Kasayama S. Clinical impact of glycated albumin as another glycemic control marker. *Endocr J* 2010; 57: 751–762.
20. Suwa T, Ohta A, Matsui T, *et al.* Relationship between clinical markers of glycemia and glucose excursion evaluated by continuous glucose monitoring (CGM). *Endocr J* 2010; 57: 135–140.
21. Pu LJ, Lu L, Shen WF, *et al.* Increased serum glycated albumin level is associated with the presence and severity of coronary artery disease in type 2 diabetic patients. *Circ J* 2007; 71: 1067–1073.
22. Santos-Oliveira R, Purdy C, da Silva MP, *et al.* Haemoglobin A1c levels and subsequent cardiovascular disease in persons without diabetes: a meta-analysis of prospective cohorts. *Diabetologia* 2011; 54: 1327–1334.
23. Selvin E, Steffes MW, Zhu H, *et al.* Glycated hemoglobin, diabetes, and cardiovascular risk in nondiabetic adults. *N Engl J Med* 2010; 362: 800–811.
24. Stratton IM, Adler AI, Neil HA, *et al.* Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *BMJ* 2000; 321: 405–412.
25. Ishizaka N, Ishizaka Y, Takahashi E, *et al.* Association between insulin resistance and carotid arteriosclerosis in subjects with normal fasting glucose and normal glucose tolerance. *Arterioscler Thromb Vasc Biol* 2003; 23: 295–301.
26. Lipska KJ, De Rekeneire N, Van Ness PH, *et al.* Identifying dysglycemic states in older adults: implications of the emerging use of hemoglobin A1c. *J Clin Endocrinol Metab* 2010; 95: 5289–5295.
27. Lin TT, Pin FJ, Tan E, *et al.* HbA1c may not be a sensitive determinant of diabetic status in the elderly. *Diabetes Res Clin Pract* 2011; 92: 31–33.
28. Christensen DL, Witte DR, Kaduka L, *et al.* Moving to an A1C-based diagnosis of diabetes has a different impact on prevalence in different ethnic groups. *Diabetes Care* 2010; 33: 580–582.
29. Davidson MB, Schriger DL. Effect of age and race/ethnicity on HbA1c levels in people without known diabetes mellitus: implications for the diagnosis of diabetes. *Diabetes Res Clin Pract* 2010; 87: 415–421.
30. Khoo J, Tay TL, Foo JP, *et al.* Sensitivity of A1C to diagnose diabetes is decreased in high-risk older Southeast Asians. *J Diabetes Complications* 2012; 26: 99–101.
31. Imai T, Oikawa Y, Shimada A. Improved monitoring of the hyperglycemic state in type 1 diabetes patients by use of the glycoalbumin/HbA1c ratio. *Rev Diabet Stud* 2007; 4: 44–48.
32. Moon JH, Chae MK, Kim KJ, *et al.* Decreased endothelial progenitor cells and increased serum glycated albumin are independently correlated with plaque-forming carotid artery atherosclerosis in type 2 diabetes patients without documented ischemic disease. *Circ J* 2012; 76: 2273–2279.
33. Song SO, Kim KJ, Lee B-W, *et al.* Serum glycated albumin predicts the progression of carotid arterial atherosclerosis. *Atherosclerosis* 2012; 225: 450–455.
34. Koga M, Matsumoto S, Saito H, *et al.* Body mass index negatively influences glycated albumin, but not glycated hemoglobin, in diabetic patients. *Endocr J* 2006; 53: 387–391.
35. Koga M, Otsuki M, Matsumoto S, *et al.* Negative association of obesity and its related chronic inflammation with serum glycated albumin but not glycated hemoglobin levels. *Clin Chim Acta* 2007; 378: 48–52.
36. Selvin E, Francis LM, Ballantyne CM, *et al.* Nontraditional markers of glycemia: associations with microvascular conditions. *Diabetes Care* 2011; 34: 960–967.

Atherosclerosis 231 (2013) 421–426

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Effect of impaired glucose tolerance on atherosclerotic lesion formation: An evaluation in selectively bred mice with different susceptibilities to glucose intolerance

Akira Asai, Mototsugu Nagao, Momoyo Kawahara, Yuki Shuto, Hitoshi Sugihara, Shinichi Oikawa*

Division of Endocrinology and Metabolism, Department of Medicine, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113-8603, Japan

ARTICLE INFO

Article history:
Received 7 June 2013
Received in revised form
17 September 2013
Accepted 7 October 2013
Available online 17 October 2013

Keywords:
Impaired glucose tolerance
Postprandial hyperglycemia
Atherosclerosis
Animal model

ABSTRACT

Objective: Impaired glucose tolerance (IGT) is an independent risk factor for atherosclerotic cardiovascular disease. However, due to the lack of appropriate animal models, the underlying mechanisms for IGT-induced atherosclerosis remain to be elucidated *in vivo*. We recently used selective breeding to establish 2 mouse lines with distinctively different susceptibilities to diet-induced glucose intolerance, designated selectively bred diet-induced glucose intolerance-resistant (SDG-R) and SDG-prone (SDG-P), respectively. Here, we assessed atherosclerotic lesion formation in these mice.

Methods: Female SDG-R and SDG-P mice were fed an atherogenic diet (AD; 1.25% cholesterol, 0.5% sodium cholate, and 36% energy as fat) for 20 weeks (8–28 weeks of age). Oral glucose tolerance tests were performed during the AD-feeding period. Atherosclerotic lesion formation was quantitatively analyzed in serial aortic sinus sections by oil red O staining. Plasma lipids were measured after the AD-feeding period.

Results: Glucose tolerance was impaired in SDG-P mice as compared to SDG-R mice over the 20-week AD-feeding period. No significant differences were observed in any plasma lipid measurement between the 2 mouse lines. Aortic sinus atherosclerotic lesion formation in SDG-P mice was approximately 4-fold greater than that in SDG-R mice.

Conclusion: In 2 mouse lines with different susceptibilities to diet-induced glucose intolerance, IGT accelerated atherosclerotic lesion formation. These mice may therefore serve as useful *in vivo* models for investigating the causal role of IGT in the pathogenesis of atherosclerosis.

1. Introduction

Individuals with diabetes have an increased risk for atherosclerotic cardiovascular events, including myocardial infarction, stroke, and peripheral vascular disease [1,2]. In individuals with pre-diabetes as well, impaired glucose tolerance (IGT) is an independent risk factor for cardiovascular disease [3–9]. Although the benefit of intensive glycemic control for improved cardiovascular outcomes did not yield statistically significant results in 2 large prospective studies (the Diabetes Chronic Complications Trial [DCCT] in young subjects with type 1 diabetes [10] and the United Kingdom Prospective Diabetes Study [UKPDS] in subjects with newly diagnosed type 2 diabetes [11]) during the original study periods, long-term follow-up of those subjects revealed that intensive glycemic control early in the course of diabetes reduced the occurrence subsequent cardiovascular events [12,13]. These intriguing observations of the so-called "legacy effect" or "metabolic memory" imply that glucose intolerance in pre-diabetes or early-stage diabetes may play a pivotal role in the initiation of the atherosclerotic process.

A number of animal models have been used to investigate the role of hyperglycemia in atherosclerosis [14,15]. The predominant models used have been genetically atherosclerosis-prone, hypercholesterolemic mice (apolipoprotein E [apoE]- or LDL receptor [LDLR]-deficient mice) in combination with chemical destruction of pancreatic β-cells (by streptozotocin) or crossbreeding with genetically obese type 2 diabetic models (*db/db* or *ob/ob*). However, severe hypercholesterolemia in apoE- or LDLR-deficient mice often masks the effect of hyperglycemia on the atherosclerotic process [16,17]. In addition, both streptozotocin-treated mice and

* Corresponding author. Tel.: +81 3 3822 2131; fax: +81 3 5802 8153.
E-mail address: shinichi@nms.ac.jp (S. Oikawa).

0021-9150/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.atherosclerosis.2013.10.009

genetically obese mice show severe hyperglycemia even under fasting conditions. Thus, these mice serve as appropriate models for established diabetes rather than for IGT early in the course of diabetes.

Recently, we established 2 mouse lines with distinctively different glucose tolerance by selective breeding. In brief, using C57BL/6, C3H, and AKR as background strains, mice exhibiting superior or inferior glucose tolerance after high-fat feeding were selectively bred and designated as selectively bred diet-induced glucose intolerance-resistant (SDG-R) and SDG-prone (SDG-P), respectively [18]. Since SDG-P mice show evident glucose intolerance as compared with SDG-R mice without any apparent difference in fasting blood glucose levels, these mice may serve as appropriate models for studying IGT-related disorders, including atherosclerotic cardiovascular complications. In this study, we assessed atherosclerotic lesion formation in these novel mouse lines.

2. Methods

2.1. Animals and diets

Female SDG-R and SDG-P mice (14th–19th generations [18]) bred at the Institute for Animal Reproduction (Kasumigaura, Japan) were used (see Supplementary Fig. S1 for detailed breeding history). Female mice are known to be more susceptible to atherosclerotic lesion formation than males [19,20]. The mice were fed a standard rodent chow (MF; Oriental Yeast, Tokyo, Japan) until 8 weeks of age. Subsequently, the diet was changed to an atherogenic diet (AD) containing 1.25% cholesterol, 0.5% sodium cholate, and 36% energy as fat (F2HFD1; Oriental Yeast: see Supplementary Table S1 for detailed composition). The mice were maintained on the AD for 20 weeks in standard housing (3 or 4 mice per cage) with a 14-h light (06:00–20:00 h)/10-h dark cycle. Food intake, body weight, and random-fed blood glucose levels were monitored every 4 weeks (at 16:00 h). Daily food intake per mouse was calculated from 1-week food consumption per cage. Circadian blood glucose levels were measured on 1 day in the 14th week of AD feeding; measurements were taken every 4 h. This study was conducted under approval from the institutional animal care and use committee of Nippon Medical School.

2.2. Oral glucose tolerance test (OGTT)

Glucose tolerance was evaluated by OGTT. At 1 week before (under standard rodent chow), 10 week after, and 19 week after the start of AD feeding, overnight-fasted mice were administered a 20% glucose solution (40, 50, and 60 mg glucose/mouse, respectively) by oral gavage. The dose was based on the average body weight at each time point (approximately 2 g/kg). Blood samples were obtained by tail bleeding. Blood glucose was measured with a glucose sensor (Glutest Neo Super; Sanwa Kagaku Kenkyusho, Nagoya, Japan). The insulin concentration of the plasma was measured by ELISA (Ultra Sensitive Mouse Insulin kit; Morinaga Institute of Biological Science, Yokohama, Japan).

2.3. Plasma lipid analysis

At the end of the 20-week AD-feeding period, blood was collected from the inferior vena cava of overnight-fasted mice under anesthesia. Total cholesterol, HDL-cholesterol (sodium phosphotungstate-magnesium chloride precipitation method), triacylglycerols, and non-esterified fatty acids in the blood plasma were measured using commercial kits (Wako Pure Chemical, Osaka, Japan).

2.4. Evaluation of atherosclerotic lesion formation

Atherosclerotic lesion formation in the aortic sinus was quantitatively analyzed based on the method of Paigen et al. [20] with modifications. After perfused *in situ* with saline followed by 4% formaldehyde in PBS from left ventricle, the heart was isolated and further fixed overnight with 4% formaldehyde in PBS. It was then cut at a plane parallel to atrial appendages and the upper part including aortic root was embedded in OCT compound (Sakura Finetek, Tokyo, Japan). Cryostat sections were cut from the left ventricular outflow tract and discarded until 3 valve cusps were shown. Then, 45 serial cross-sections (10-μm thickness) of aortic sinus were prepared (i.e., covered a distance of 450 μm). Of the 45 serial sections, every 5 sections (total of 9 sections each separated by 50 μm) were stained with oil red O and counterstained with hematoxylin. The oil red O-stained sections were examined under a light microscope (AX80; Olympus, Tokyo, Japan) with cellSens imaging software (ver. 1.4.1; Olympus). The oil red O-stained area was determined manually from the photomicrograph images [21] on Photoshop Elements software (ver. 9.0.3; Adobe Systems, San Jose, CA). For each mouse, the oil red O-stained area of the 9 sections was averaged and expressed as the mean lesion size.

Immunohistochemical staining was performed to confirm macrophage infiltration into the atherosclerotic lesions. In brief, serial sections to the oil red O-stained ones were stained with MOMA-2 rat monoclonal antibody to mouse macrophages (AbD Serotec, Oxford, UK) using Vectastain Elite ABC kit (Vector, Burlingame, CA) followed by hematoxylin counter stain.

2.5. Statistical analysis

Values are presented as mean ± SEM. Values of $p < 0.05$ by Student's *t*-test were considered statistically different between the SDG-R and SDG-P groups.

3. Results

3.1. Body weight, food intake, and tissue weight

Over the 20-week AD-feeding period, SDG-P mice had a greater body weight than SDG-R mice (Fig. 1A). Food intake of SDG-P mice was also higher than that of SDG-R mice during most of the feeding period (Fig. 1B). At the end of the feeding period, SDG-P mice showed a greater gonadal fat mass as compared with SDG-R mice, whereas no differences were observed in liver weight (Table 1).

3.2. Glucose tolerance and random-fed blood glucose

In the OGTT, no significant differences were observed in fasting blood glucose levels between the SDG-R and the SDG-P mouse groups at 1 week before, 10 weeks after, and 19 weeks after the start of AD feeding (Fig. 2A–C; 0 min). However, SDG-P mice showed higher blood glucose levels at 30 min and subsequent times after glucose injection as compared with SDG-R mice during the 20-week AD-feeding period (Fig. 2A–C). Although a statistical difference was observed in the fasting insulin levels before the start of AD feeding, no such differences were observed in post-challenge insulin levels over the feeding period (Fig. 2E–G).

Random-fed blood glucose levels were higher in SDG-P mice than in SDG-R mice over the AD-feeding period (Fig. 3A). In addition, the circadian blood glucose profile revealed that SDG-P mice had higher blood glucose levels throughout the day with greater fluctuations than those seen in SDG-R mice (Fig. 3B). The standard deviations of individual circadian blood glucose levels (6 time

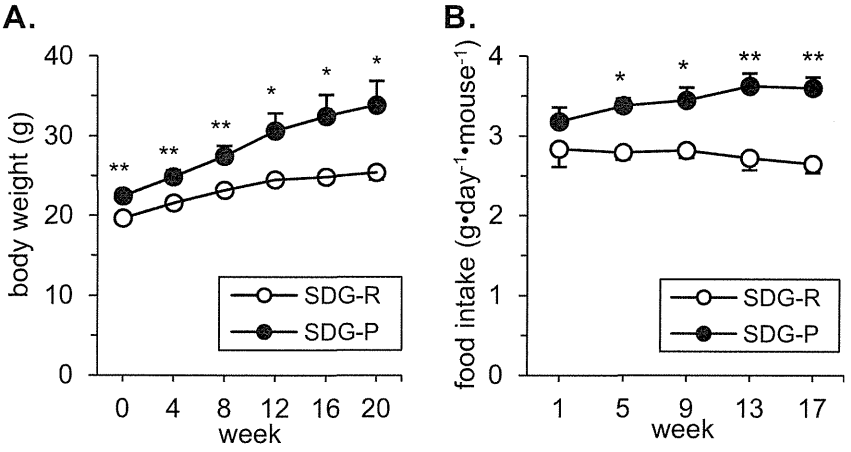

Fig. 1. Body weight gain (A) and food intake (B) of glucose intolerance-resistant (SDG-R) and prone (SDG-P) mice during atherogenic diet feeding. Data are expressed as mean ± SEM (A, $n = 10–13$ mice; B, $n = 4$ cages). $^{*}p < 0.05$, $^{**}p < 0.01$.

points) were 0.60 ± 0.09 and 1.01 ± 0.12 mmol/l in the SDG-R and SDG-P groups, respectively ($p = 0.013$, $n = 5–6$).

3.3. Plasma lipid profile

Table 1 shows the plasma lipid profiles of the SDG-R and the SDG-P mouse groups at the end of the 20-week AD-feeding period. No significant differences were observed in total cholesterol, HDL-cholesterol, non-HDL-cholesterol, triacylglycerols, or non-esterified fatty acid levels between the 2 mouse lines.

3.4. Atherosclerotic lesion formation

At the end of the 20-week AD-feeding period, lipid-laden plaque formation was observed in the intimal area of the aortic sinus (Fig. 4A–D). The lesion formation was confirmed by the immunohistochemical detection of macrophage infiltration, another hallmark of atherosclerotic lesions (Fig. 4E–H). Atherosclerotic lesion formation in SDG-P mice was approximately 4-fold greater than that in SDG-R mice (Fig. 4I).

4. Discussion

IGT has been postulated to play an important role in the pathogenesis of atherosclerosis, but few animal models are available to investigate the causal mechanisms appropriately. Existing diabetic models display severe hyperglycemia, even in fasting conditions. In addition, the severe hyperglycemia is often accompanied by dyslipidemia [16,17]. The lipid abnormalities in diabetes make it difficult to dissociate the effects of hyperglycemia *per se* from those of dyslipidemia.

Table 1
Relative tissue weights and plasma lipid profiles of glucose intolerance-resistant (SDG-R) and prone (SDG-P) mice after atherogenic diet feeding.

	SDG-R ($n = 12$)	SDG-P ($n = 11$)	p value[a]
Tissue weight, mg/g body weight			
Liver	78.7 ± 5.0	67.1 ± 3.4	0.070
Gonadal fat	13.2 ± 1.7	27.1 ± 4.2	0.009
Plasma lipids, mmol/l			
Total-cholesterol	4.36 ± 0.33	4.36 ± 0.73	1.00
HDL-cholesterol	1.32 ± 0.34	0.74 ± 0.11	0.13
Non-HDL-cholesterol	3.03 ± 0.26	3.62 ± 0.65	0.42
Triacylglycerols	0.39 ± 0.04	0.43 ± 0.05	0.50
Non-esterified fatty acids	0.77 ± 0.07	0.72 ± 0.07	0.67

Values are expressed as mean ± SEM.
[a] Student's *t*-test.

In contrast to existing diabetic models, the present results demonstrate that SDG-P mice have the characteristics of a useful IGT model (modest hyperglycemia under post-challenge or random-fed conditions, but not fasting conditions), while SDG-R mice provide a normal glucose-tolerant control. Fasting blood glucose levels in SDG-P mice tented to be increased during the AD-feeding period most probably due to the insulin resistance arising from increased fat deposition (see Supplementary Fig. S2 for the results of insulin tolerance test). At 19 weeks after the start of AD feeding, fasting blood glucose levels of SDG-R and SDG-P were 3.84 ± 0.26 ($n = 6$) and 8.26 ± 1.51 ($n = 4$), respectively ($p = 0.07$; Fig. 2C); however, the levels were still far lower than those of existing diabetes models such as streptozotocin-treated mice or *db/db* mice.

In addition, no significant differences in the lipid profiles were observed between the 2 mouse lines. Even under the AD feeding, these mice did not display overt hypercholesterolemia. Due to the overwhelming impact of cholesterol on atherosclerosis, extremely high levels of non-HDL-cholesterol in apoE- or LDLR-deficient mice can hamper any attempt to isolate the effect of hyperglycemia [16,17]. In contrast to the hypercholesterolemic mice in the abovementioned study, plasma lipid levels in our mice were almost comparable to the normal range in humans. Thus, our mouse lines will be appropriate models for defining the effect of glucose intolerance without the concomitant effect of overt dyslipidemia. However, more detailed lipoprotein fraction analysis may be needed to clarify the lipoprotein distributions. In patients with type 2 diabetes, lipoprotein abnormalities in the subclasses (in the size and function) have been discussed in relation to the increased cardiovascular risk [22]. Furthermore, a most recent report demonstrated that the subclass abnormalities already exist in individuals with IGT [23]. Hence, the subclass analysis may be useful in understanding the IGT-related lipoprotein abnormalities and their possible involvement in the accelerated lesion formation in SDG-P mice.

SDG-P mice with IGT were more susceptible to atherosclerotic lesion formation than glucose-tolerant SDG-R mice. Because our mice are not genetically engineered to form overt atherosclerotic lesions, the lesion formation was much less than that in apoE- or LDLR-deficient mice. Our mice are therefore not suitable for studying the advanced-stage of atherosclerotic disorders, but will be appropriate for investigating early stage atherogenesis. As

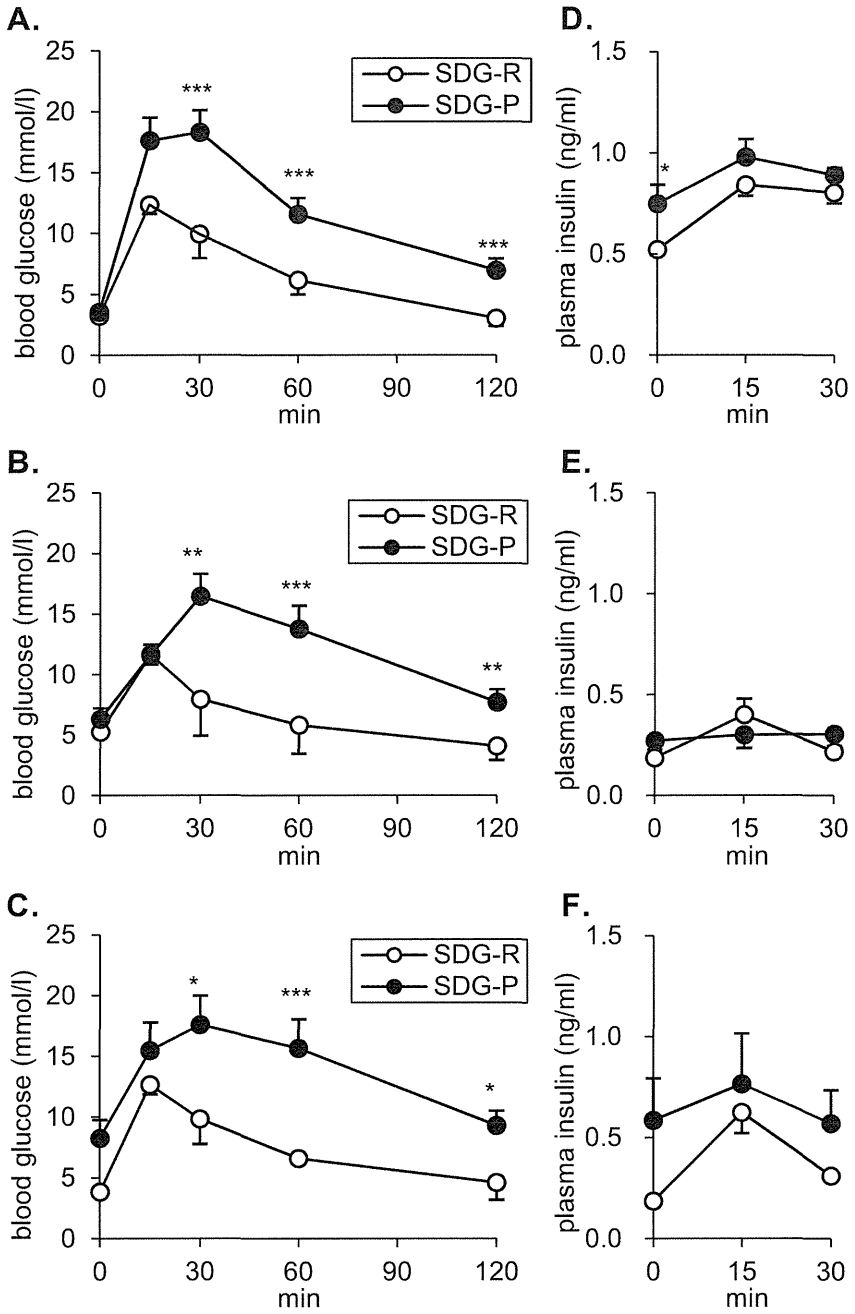

Fig. 2. Blood glucose (A–C) and plasma insulin (D–F) levels of glucose intolerance-resistant (SDG-R) and prone (SDG-P) mice in OGTT at 1 week before (A and D; 40 mg glucose/mouse), 10 weeks after (B and E; 50 mg glucose/mouse), and 19 weeks after (C and F; 60 mg glucose/mouse) the start of atherogenic diet feeding. Data are expressed as mean ± SEM of 4–6 mice. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

demonstrated in follow-up studies of the DCCT and UKPDS trials [12,13], glycemic control in early stage diabetes is important to prevent subsequent cardiovascular complications. This strongly suggests that IGT can modify early stage atherogenesis, which eventually leads to increased incidence of and mortality from cardiovascular events. Renard et al. [24] demonstrated in LDLR-deficient mice with viral β-cell destruction that hyperglycemia leads to the initiation of atherosclerotic lesions, whereas advanced lesion formation is largely depend on diabetes-induced dyslipidemia. They also elucidated the distinct effect of hyperglycemia on early stage atherosclerosis; however, their diabetic mice had both severe hyperglycemia (~20 mmol/l) and hypercholesterolemia (~9 mmol/l), even under cholesterol-free diet feeding [24]. Moderately hyperglycemic SDG-P mice without apparent dyslipidemia will be preferable for studying the causal relationship between pre-diabetes or early stage diabetes and the pathogenesis of atherosclerosis. Recently, Bartels et al. [25] also reported that prolonged feeding (12 months) of high-fat diet (60% energy as fat) led to a high incidence (64%) of small atherosclerotic lesions in C57BL/6 mice in coincidence with glucose intolerance but without overt dyslipidemia.

Since considerable attention has been focused on the epidemiological impact of IGT on cardiovascular complications, a number of studies have reported the possible roles of glucose fluctuations, a hallmark of IGT, in atherogenesis. For example, intermittent high glucose exposures were demonstrated to induce oxidative stress

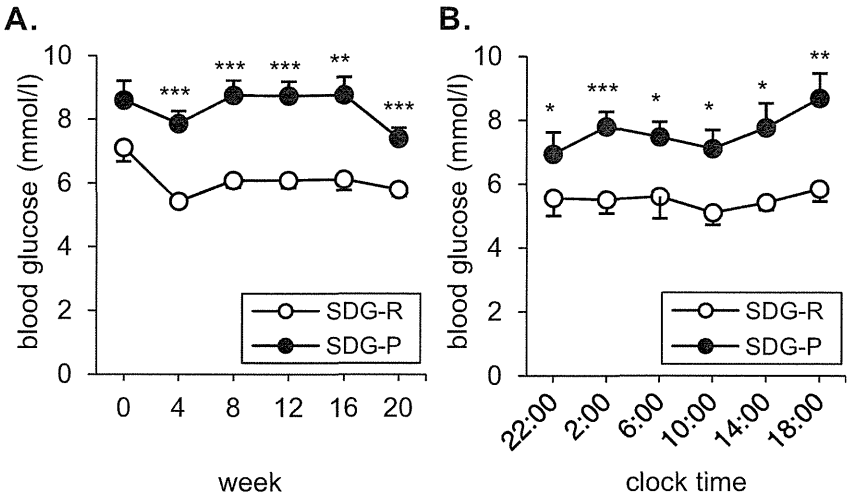

Fig. 3. Random-fed blood glucose levels and circadian blood glucose profiles in glucose intolerance-resistant (SDG-R) and prone (SDG-P) mice. (A) Random-fed blood glucose levels were measured every 4 weeks at 16:00 h during atherogenic diet feeding. (B) Circadian blood glucose profiles were obtained from measurements taken at 4-h intervals on 1 day in the 14th week of atherogenic diet feeding. Data are expressed as mean ± SEM (A, $n = 10–13$; B, $n = 5–6$). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

and subsequently produce various pro-atherogenic changes in cultured vascular endothelial cells, smooth muscle cells, and monocytes/macrophages (e.g., augmented expressions of adhesion molecules and inflammatory cytokines) [26]. Transient hyperglycemia-induced oxidative stress was also demonstrated in humans [27]. In the present study, SDG-P mice had greater circadian variations in random-fed blood glucose levels as compared with SDG-R mice, suggesting that the fluctuations of blood glucose, in addition to the mean levels, were likely to be involved in the accelerated lesion formation observed in SDG-P mice. Hence, these mice may contribute to a better understanding of the cellular and molecular mechanisms that are responsible *in vivo* for IGT-induced atherogenesis. For example, biochemical and histological analyses of the causal mechanisms (e.g., macrophage infiltration into and adhesion molecule expression on the aortic wall) at earlier time points are now of great interest.

Because SDG-R and SDG-P mice are outbred lines from 3 inbred strains (C57BL/6J, C3H/HeJ, and AKR/N) [18], our mice harbor certain genetic variations. The genetic diversity was a key factor in the wide distribution of glucose tolerance, which enabled us to establish the 2 mouse lines. At the same time, although glucose tolerance was the sole criterion for the selective breeding, we cannot exclude the possibility that other genetic factors, which can affect atherogenesis independent of blood glucose levels, were concomitantly selected for in the SDG-R and SDG-P mouse lines. Among the 3 background strains, C3H and AKR are known to be highly resistant to atherosclerotic lesion formation, whereas C57BL/6 is relatively prone to that [19,28,29]. The genetic diversity in susceptibility to atherosclerosis may partially account for the different lesion size between SDG-R and SDG-P independent of blood glucose levels. For example, although it remains controversial [30], a defective allele of Toll-like receptor 4 (*Tlr4*) in C3H/HeJ has been implicated in the resistant phenotype to atherosclerosis [31]. Unexpectedly, however, the defective *Tlr4* allele was found only in atherosclerosis-prone SDG-P mice, but not in SDG-R (see Supplementary Fig. S3 for the results of genotyping). Hence, the

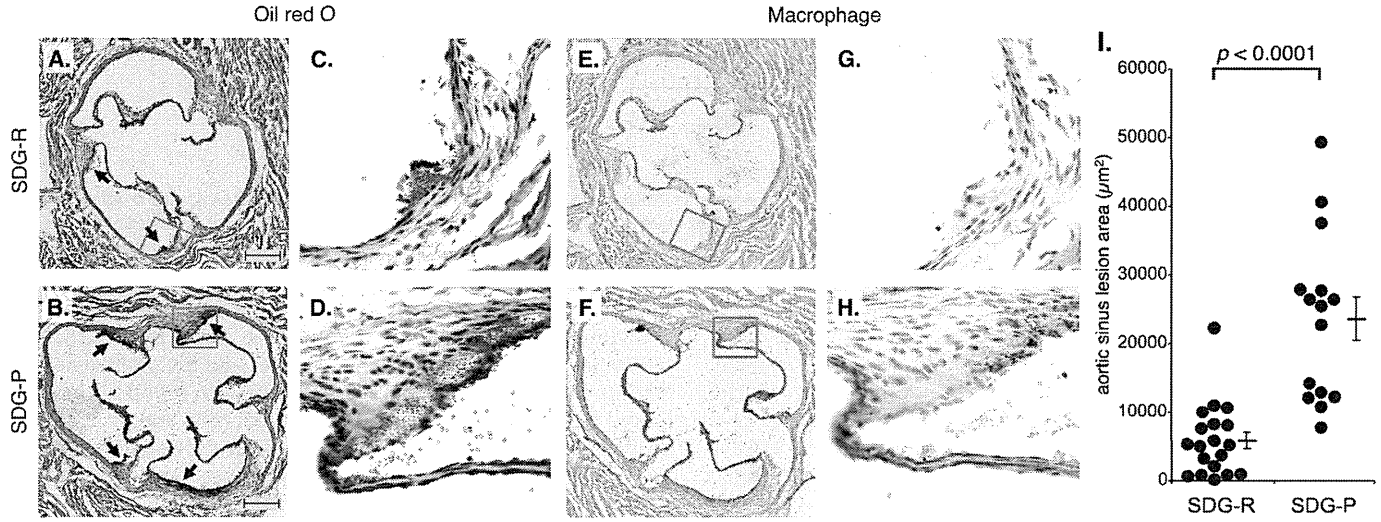

Fig. 4. Atherosclerotic lesion formation in the aortic sinus of glucose intolerance-resistant (SDG-R) and prone (SDG-P) mice after atherogenic diet feeding. (A, B) Representative images of oil red O-stained atherosclerotic lesions in the aortic sinus (arrows). Bar: 200 μm (C, D) Higher magnification images from the fields indicated by squares in *panels A and B.* (E, F) Immunohistochemical staining for macrophages (brown) in the serial cross-sections to the corresponding oil-red O-stained images. (G, H) Higher magnification images from the fields indicated by squares in *panels E and F.* (I) Quantitative analysis of the lesion area. Each dot indicates mean lesion size in each mouse. Bars indicate mean ± SEM of 15–19 mice.

atherosclerosis-resistant phenotype of SDG-R mice should not be the result of defective TLR4 signaling. A polymorphism in vascular cell adhesion molecule 1 (*Vcam1*) among the 3 background strains is also reported to affect the development of atherosclerosis [32,33]. The polymorphism of *Vcam1* was found in both mouse lines (Supplementary Fig. S3). In any case of *Tlr4* or *Vcam1* genotypes inherited in both mouse lines, however, SDG-P mice showed greater lesion formation than SDG-R mice carrying the same alleles. In addition, the hyperphagic behavior and consequent greater weight gain observed in the SDG-P as compared with the SDG-R mice might be determined by factors independent of glucose intolerance. Further research into such genetic variations will strengthen the value of the novel mouse lines as IGT-induced atherosclerosis model animals.

In this study, a cholate-containing AD was used to ensure the atherosclerotic lesion formation in wild-type mice. Besides the critical role in facilitating intestinal cholesterol absorption, bile acids can modulate diverse signaling pathways in metabolic homeostasis and inflammation [34–36]. The potential pleiotropic effects of exogenous cholate may therefore be taken into account in interpreting the present results.

In conclusion, the present results demonstrate that glucose intolerance-prone SDG-P mice are more susceptible to atherosclerotic lesion formation than glucose-intolerance resistant SDG-R mice. The novel mouse lines with different susceptibilities to glucose intolerance may therefore serve as useful animal models for studying the pathogenesis, prevention, and treatment of IGT-induced atherosclerotic disorders.

Conflicts of interest

The authors declare that there is no duality of interest associated with this manuscript.

Acknowledgments

This study was supported in part by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (to MN), a grant from Lilly Grant Office (to AA), and a grant from Japan Metabolic Syndrome Study Group (to MN).

Appendix A. Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.atherosclerosis.2013.10.009.

References

[1] Ruderman NB, Haudenschild C. Diabetes as an atherogenic factor. Prog Cardiovasc Dis 1984;26:373–412.
[2] Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. JAMA 2002;287:2570–81.
[3] Fujishima M, Kiyohara Y, Kato I, et al. Diabetes and cardiovascular disease in a prospective population survey in Japan: The Hisayama Study. Diabetes 1996;45:S14–6.
[4] Rodriguez BL, Curb JD, Burchfiel CM, et al. Impaired glucose tolerance, diabetes, and cardiovascular disease risk factor profiles in the elderly. The Honolulu Heart Program. Diabetes Care 1996;19:587–90.
[5] Tominaga M, Eguchi H, Manaka H, Igarashi K, Kato T, Sekikawa A. Impaired glucose tolerance is a risk factor for cardiovascular disease, but not impaired fasting glucose. The Funagata Diabetes Study. Diabetes Care 1999;22:920–4.
[6] Balkau B, Shipley M, Jarrett RJ, et al. High blood glucose concentration is a risk factor for mortality in middle-aged nondiabetic men 20-year follow-up in the Whitehall Study, the Paris Prospective Study, and the Helsinki Policemen Study. Diabetes Care 1998;21:360–7.
[7] de Vegt F, Dekker JM, Ruhé HG, et al. Hyperglycaemia is associated with all-cause and cardiovascular mortality in the Hoorn population: the Hoorn Study. Diabetologia 1999;42:926–31.
[8] The DECODE Study Group. Glucose tolerance and cardiovascular mortality: comparison of fasting and 2-hour diagnostic criteria. Arch Intern Med 2001;161:397–405.
[9] Meigs JB, Nathan DM, D'Agostino RB, Wilson PWF., Framingham Offspring Study. Fasting and postchallenge glycemia and cardiovascular disease risk: the Framingham Offspring Study. Diabetes Care 2002;25:1845–50.
[10] The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993;329:977–86.
[11] UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet 1998;352:837–53.
[12] The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. N Engl J Med 2005;353:2643–53.
[13] Holman R, Paul S, Bethel M, Matthews D, Neil H. 10-year follow-up of intensive glucose control in type 2 diabetes. N Engl J Med 2008;359:1577–89.
[14] Goldberg IJ, Dansky HM. Diabetic vascular disease: an experimental objective. Arterioscler Thromb Vasc Biol 2006;26:1693–701.
[15] Wu K, Huan Y. Diabetic atherosclerosis mouse models. Atherosclerosis 2007;191:241–9.
[16] Kanter JE, Johansson F, LeBoeuf RC, Bornfeldt KE. Do glucose and lipids exert independent effects on atherosclerotic lesion initiation or progression to advanced plaques? Circ Res 2007;100:769–81.
[17] Chait A, Bornfeldt K. Diabetes and atherosclerosis: is there a role for hyperglycemia? J Lipid Res 2009;50(Suppl.):S335–9.
[18] Nagao M, Asai A, Kawahara M, et al. Selective breeding of mice for different susceptibilities to high fat diet-induced glucose intolerance: development of two novel mouse lines, Selectively bred Diet-induced Glucose intolerance-Prone and -Resistant. J Diabetes Invest 2012;3:245–51.
[19] Paigen B, Holmes PA, Mitchell D, Albee D. Comparison of atherosclerotic lesions and HDL-lipid levels in male, female, and testosterone-treated female mice from strains C57BL/6, BALB/c, and C3H. Atherosclerosis 1987;64:215–21.
[20] Paigen B, Morrow A, Holmes PA, Mitchell D, Williams RA. Quantitative assessment of atherosclerotic lesions in mice. Atherosclerosis 1987;68:231–40.
[21] Tangirala RK, Rubin EM, Palinski W. Quantitation of atherosclerosis in murine models: correlation between lesions in the aortic origin and in the entire aorta, and differences in the extent of lesions between sexes in LDL receptor-deficient and apolipoprotein E-deficient mice. J Lipid Res 1995;36:2320–8.
[22] Krauss R. Lipids and lipoproteins in patients with type 2 diabetes. Diabetes Care 2004;27:1496–504.
[23] Lorenzo C, Hartnett S, Hanley AJ, et al. Impaired fasting glucose and impaired glucose tolerance have distinct lipoprotein and apolipoprotein changes: the Insulin Resistance Atherosclerosis Study. J Clin Endocrinol Metab 2013;98:1622–30.
[24] Renard C, Kramer F, Johansson F, et al. Diabetes and diabetes-associated lipid abnormalities have distinct effects on initiation and progression of atherosclerotic lesions. J Clin Invest 2004;114:659–68.
[25] Bartels ED, Bang CA, Nielsen LB. Early atherosclerosis and vascular inflammation in mice with diet-induced type 2 diabetes. Eur J Clin Invest 2009;39:190–9.
[26] Ceriello A. Postprandial hyperglycemia and diabetes complications: is it time to treat? Diabetes 2005;54:1–7.
[27] Ceriello A, Esposito K, Piconi L, et al. Oscillating glucose is more deleterious to endothelial function and oxidative stress than mean glucose in normal and type 2 diabetic patients. Diabetes 2008;57:1349–54.
[28] Paigen B, Morrow A, Brandon C, Mitchell D, Holmes P. Variation in susceptibility to atherosclerosis among inbred strains of mice. Atherosclerosis 1985;57:65–73.
[29] Paigen B, Ishida BY, Verstuyft J, Winters RB, Albee D. Atherosclerosis susceptibility differences among progenitors of recombinant inbred strains of mice. Arteriosclerosis 1990;10:316–23.
[30] Wang SS, Shi W, Wang X, et al. Mapping, genetic isolation, and characterization of genetic loci that determine resistance to atherosclerosis in C3H mice. Arterioscler Thromb Vasc Biol 2007;27:2671–6.
[31] Michelsen KS, Wong MH, Shah PK, et al. Lack of toll-like receptor 4 or myeloid differentiation factor 88 reduces atherosclerosis and alters plaque phenotype in mice deficient in apolipoprotein E. Proc Natl Acad Sci U S A 2004;101:10679–84.
[32] Pei H, Wang Y, Miyoshi T, et al. Direct evidence for a crucial role of the arterial wall in control of atherosclerosis susceptibility. Circulation 2006;114:2382–9.
[33] Miyoshi T, Yuan Z, Shi W. Association of a *Vcam1* mutation with atherosclerosis susceptibility in diet-induced models of atherosclerosis. Atherosclerosis 2008;196:234–9.
[34] Houten SM, Watanabe M, Auwerx J. Endocrine functions of bile acids. EMBO J 2006;25:1419–25.
[35] Nguyen A, Bouscarel B. Bile acids and signal transduction: role in glucose homeostasis. Cell Signal 2008;20:2180–97.
[36] Vergnes L, Phan J, Strauss M, Tafuri S, Reue K. Cholesterol and cholate components of an atherogenic diet induce distinct stages of hepatic inflammatory gene expression. J Biol Chem 2003;278:42774–84.

PLOS ONE

Characterization of Pancreatic Islets in Two Selectively Bred Mouse Lines with Different Susceptibilities to High-Fat Diet-Induced Glucose Intolerance

Mototsugu Nagao[1], Akira Asai[1], Wataru Inaba[2], Momoyo Kawahara[1], Yuki Shuto[1], Shunsuke Kobayashi[1], Daisuke Sanoyama[1], Hitoshi Sugihara[1], Soroku Yagihashi[2], Shinichi Oikawa[1]*

1 Department of Endocrinology, Diabetes and Metabolism, Graduate School of Medicine, Nippon Medical School, Tokyo, Japan, **2** Department of Pathology and Molecular Medicine, Hirosaki University Graduate School of Medicine, Hirosaki, Japan

Abstract

Hereditary predisposition to diet-induced type 2 diabetes has not yet been fully elucidated. We recently established 2 mouse lines with different susceptibilities (resistant and prone) to high-fat diet (HFD)-induced glucose intolerance by selective breeding (designated selectively bred diet-induced glucose intolerance-resistant [SDG-R] and -prone [SDG-P], respectively). To investigate the predisposition to HFD-induced glucose intolerance in pancreatic islets, we examined the islet morphological features and functions in these novel mouse lines. Male SDG-P and SDG-R mice were fed a HFD for 5 weeks. Before and after HFD feeding, glucose tolerance was evaluated by oral glucose tolerance test (OGTT). Morphometry and functional analyses of the pancreatic islets were also performed before and after the feeding period. Before HFD feeding, SDG-P mice showed modestly higher postchallenge blood glucose levels and lower insulin increments in OGTT than SDG-R mice. Although SDG-P mice showed greater β cell proliferation than SDG-R mice under HFD feeding, SDG-P mice developed overt glucose intolerance, whereas SDG-R mice maintained normal glucose tolerance. Regardless of whether it was before or after HFD feeding, the isolated islets from SDG-P mice showed impaired glucose- and KCl-stimulated insulin secretion relative to those from SDG-R mice; accordingly, the expression levels of the insulin secretion-related genes in SDG-P islets were significantly lower than those in SDG-R islets. These findings suggest that the innate predispositions in pancreatic islets may determine the susceptibility to diet-induced diabetes. SDG-R and SDG-P mice may therefore be useful polygenic animal models to study the gene–environment interactions in the development of type 2 diabetes.

Citation: Nagao M, Asai A, Inaba W, Kawahara M, Shuto Y, et al. (2014) Characterization of Pancreatic Islets in Two Selectively Bred Mouse Lines with Different Susceptibilities to High-Fat Diet-Induced Glucose Intolerance. PLoS ONE 9(1): e84725. doi:10.1371/journal.pone.0084725

Editor: Pratibha V. Nerurkar, College of Tropical Agriculture and Human Resources, University of Hawaii, United States of America

Received August 16, 2013; **Accepted** November 18, 2013; **Published** January 13, 2014

Funding: This study was supported in part by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science, a Grant-in-Aid for Medical Research from Nippon Medical School, a grant from the Lilly Grant Office, and a grant from the Japan Metabolic Syndrome Study Group. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: In addition, although the authors received funding from a commercial source (Lilly), they declare this does not alter their adherence to all the PLOS ONE policies on sharing data and materials.

* E-mail: shinichi@nms.ac.jp

Introduction

Gene–environment interactions play a crucial role in the development of type 2 diabetes. For analyzing genetic factors, the polygenic background of selectively bred animal models has been investigated [1]. For instance, the Goto-Kakizaki (GK) rat [2] and Nagoya-Shibata-Yasuda (NSY) mouse [3] are non-obese diabetic models produced by repetitive selective breeding for impaired glucose metabolism. Through the analyses of these polygenic rodent models, increasing numbers of candidate genes for the pathogenesis of type 2 diabetes have been identified, most of which well resemble the genetic basis of type 2 diabetes in humans [1,4,5].

Contemporary environmental factors (*e.g.*, nutritional excess and sedentary lifestyle) cause obesity, which leads to insulin resistance in peripheral tissue [6,7]. However, not all obese individuals with insulin resistance develop type 2 diabetes because the functional and morphological compensation capacities of β cells against insulin resistance vary between individuals [8]. In rodent models, animals with high-fat diet (HFD)-induced obesity are chiefly used for assessing the impact of excess dietary fat as an environmental factor. However, the propensity for developing diet-induced diabetes varies widely even in a single strain [9]. Thus, individual differences in susceptibility to environmental factors are postulated to be determined by genetic factors. Existing polygenic models, which develop diabetes spontaneously [10], may therefore not be always appropriate to investigate the predisposition to lifestyle-related disorders because environmental factors had not been taken into account in their selective breeding.

To establish novel rodent models that can mimic the gene–environment interactions in the development of type 2 diabetes, we have performed a selective breeding of mice. In brief, using 3 inbred strains (C57BL/6, C3H, and AKR) as background, mice exhibiting superior and inferior glucose tolerance after HFD feeding have been bred repetitively to establish 2 distinct mouse lines with different susceptibilities (resistant and prone) to HFD-induced glucose intolerance, designated selectively bred diet-induced glucose intolerance-resistant (SDG-R) and -prone (SDG-