Case report

A 50-year-old obese woman with T2DM was admitted to the hospital. She was diagnosed with T2DM at the age of 38 years. IIT was started at the age of 40 years; however, her glycemic levels were very high and her average glycosylated hemoglobin (HbA1c (National Glycohemoglobin Standardization Program (NGSP) equivalent value)) level was greater than 10%. Body weight had also increased by 10 kg over 5 years (from 56 to 66.5 kg). She was hospitalized to undergo liraglutide treatment. Before hospitalization, she has been treated with four daily insulin injections (three injections of insulin lispro before breakfast (7 U), lunch (6 U), and dinner (6 U) and one of insulin NPH (20 U) at bedtime) + pioglitazone 7.5 mg/day + miglitol 150 mg/day + metformin 750 mg/day.

Physiological findings on admission

Her height was 153.6 cm and weight was 66.5 kg (body mass index (BMI) 28.2). Resting blood pressure was 111/79 mmHg. No abnormal findings were observed in the thoracoabdominal region. The vibratory sensitivity of the bilateral lower limbs and bilateral ankle reflex had decreased. She had bilateral simple retinopathy.

Laboratory findings

Laboratory data are shown in Supplementary Table S1. No abnormal findings were observed, except glucose (3+) in urinalysis. Blood cell count was normal. Levels of transaminases, creatinine, electrolytes, and blood urea nitrogen (BUN) were within normal ranges. HbA1c level was as high as 11.2% (NGSP equivalent value) and urinary C-peptide level was as low as 44 μg/day. Plasma glucose level at 8:00 and 10:00 am were 180 and 222 mg/dl, respectively. Plasma C-peptide level at 8:00 and 10:00 am were 1.03 and 1.63 ng/ml, respectively. Abdominal computed tomography (CT) revealed diffuse fatty changes in the liver.

Clinical course

Metformin 1000 mg/day, miglitol 150 mg/day, insulin lispro 20 U/day, and insulin NPH 14 U/day were started after hospitalization, in addition to diet therapy (1200 kcal). After we confirmed that the capacity of insulin secretion had decreased but was preserved, and that fasting hyperglycemia at breakfast was normalized, we changed IIT to liraglutide + glimepiride therapy in addition to metformin and miglitol administration (Fig. 1).

After day 16, her appetite was lost, an unpleasant sweet taste was experienced, and metformin and miglitol

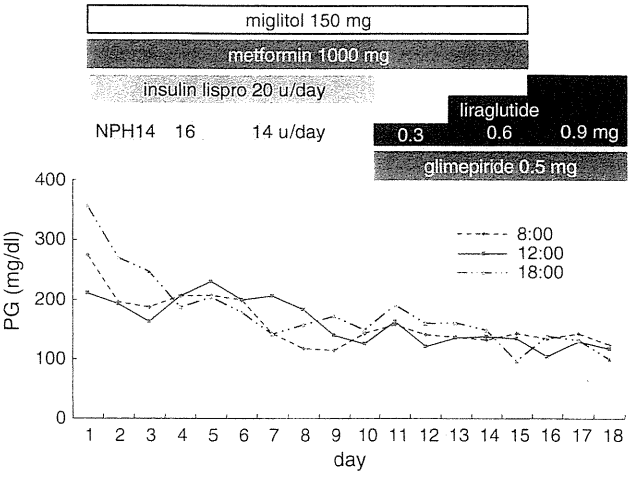

Fig. 1 Clinical course of the patient. Intensive insulin therapy was stopped on day 10, and liraglutide and 0.3 mg glimeperide were started that evening. Liraglutide was dosed up to 0.3 mg every 3 days and continued. Then 150 mg miglitol and 1000 mg metformin was stopped on day 15

administration were stopped; however, her blood glucose did not worsen and these symptoms were improved. Plasma glucose levels at 08:00/10:00/12:00/14:00/18:00/20:00/24:00/03:00 were 180/222/193/186/271/217/257/199 mg/dl, 101/157/164/110/146/192/128/90 mg/dl, and 123/188/130/169/126/150/116/113 mg/ml on days 2, 9, and 18 after hospitalization, respectively (Supplementary Figure S1). M values (M = average ($|10 \times \mathrm{Log}_{10}(\mathrm{BS}/100)|^3$) on days 2, 9, and 18, which reflect blood glucose variations during the day, were 37.3, 5.12, and 4.83, respectively [4]. Plasma C-peptide immunoreactivity (CPR) at 8:00 am/10:00 am on days 2, 9, 12, 15, and 18 was 1.03/1.63, 0.76/1.65, 1.61/2.71, 2.06/2.92, and 2.05/4.14 ng/ml, respectively (Table 1). We measured the proinsulin-to-CPR ratio to evaluate the extent of pancreatic beta cell failure [5]. This ratio on days 9, 12, 15, and 18 was 0.058, 0.031, 0.028, and 0.030, respectively. Thus, pancreatic beta cell failure had improved (Table 1). GLP-1 analogs inhibit postprandial glucagon secretion in vivo [2, 3]. Therefore, we next checked plasma glucagon concentration before and after introducing liraglutide therapy. Plasma glucagon concentrations at 8:00 am/10:00 am on days 2, 9, 12, 15, and 18 were 42/45, 62/61, 24/58, 45/94, and 20/38, respectively (Table 1).

GLP-1 analogs reportedly reduce body weight by reducing appetite [2, 3]. Finally, we checked plasma leptin and adiponectin concentrations in addition to measuring body weight. Body weight on days 2, 9, 12, 15, and 18 was 66.5, 65.5, 64.0, 63.5, and 61.8 kg, respectively (Table 1). Consistent with this result, plasma leptin concentrations on days 9, 12, 15, and 18 were 15.8, 7.5, 6.9, and 6.6 ng/ml, respectively (Table 1). Conversely, plasma adiponectin concentrations on days 9, 12, and 15 were 3.6, 3.5, and 3.1 μg/ml, respectively (Table 1). The patient was

Table 1 The effect of liraglutide on islets function, body weight, and adipokines

	Insulin Day 2		Insulin Day 9		Liraglutide 0.3 mg Day 12		Liraglutide 0.6 mg Day 15		Liraglutide 0.9 mg Day 19	
	8:00	10:00	8:00	10:00	8:00	10:00	8:00	10:00	8:00	10:00
PG (mg/dl)	180	222	101	157	134	152	129	162	123	188
S-CPR (ng/ml)	1.03	1.63	0.76	1.65	1.61	2.71	2.06	2.92	2.05	4.14
Proinsulin (pmol/l)	ND	ND	14.5	ND	16.7	ND	18.8	ND	20.4	ND
Proinsulin/CPR	ND	ND	0.058	ND	0.031	ND	0.028	ND	0.03	ND
Glucagon (pg/ml)	42	45	62	61	24	58	45	94	20	38
Abdominal circumference (cm)	100	ND	97	ND	97	ND	95.2	ND	95	ND
Body weight (kg)	66.45	ND	65.5	ND	64.0	ND	63.5	ND	61.8	ND
Leptin (ng/ml)	ND	ND	15.8	ND	7.5	ND	6.9	ND	6.6	ND
Adiponectin (μg/ml)	ND	ND	3.6	ND	3.5	ND	3.1	ND	ND	ND

subsequently discharged from the hospital on day 19. One month after discharge, her HbA1c level had improved to 8.2% (NGSP equivalent value).

Discussion

In this case, we observed that normalizing fasting hyperglycemia is important to introducing liraglutide therapy. Moreover, administration of only 0.3 mg liraglutide was sufficient to improve plasma glucose, augment glucose-stimulated insulin secretion, improve pancreatic beta cell failure, and reduce plasma leptin concentration.

We hypothesized that it was important to normalize fasting hyperglycemia to successfully introduce liraglutide therapy. The supporting evidence is:

1. increasing fasting plasma glucose concentration causes a decrease in glucose augmentation of insulin secretion [6];
2. restoration of normoglycemia with insulin in patients with T2DM improves beta cell function [7, 8];
3. normalizing hyperglycemia partly restores reduced GLP-1 and GIP receptor expression in pancreatic beta cells [9, 10]; and
4. 4-week normalization of plasma glucose level improves GLP-1 and GIP sensitivity in pancreatic beta cells in humans [11, 12].

To maintain fasting normoglycemia, we administered 0.5 mg glimepiride in the evening and metformin at bedtime rather than insulin NPH at bedtime to suppress hepatic gluconeogenesis through the night; plasma glycemic control was stable throughout liraglutide treatment. Thus, normalizing fasting hyperglycemia is beneficial for introducing liraglutide therapy.

Liraglutide is known to improve proinsulin-to-CPR ratio and fasting CPR/FPG ratio [13]. Consistent with these results, liraglutide successfully improved proinsulin-to-CPR ratio and CPR index in our patient also. Conversely, glimepiride increases both CPR and proinsulin secretion, and worsens beta cell function (proinsulin/insulin ratio) [14]. Ideally, combination therapy of long acting insulin and liraglutide will be more beneficial for this patient.

Excess glucagon secretion is important in promoting both basal and postprandial hyperglycemia in patients with T2DM [3, 15]. Some groups have shown an inhibitory effect of GLP-1 on glucagon release in patients with T2DM and in patients with type 1 diabetes [13, 16]. In contrast, we found that liraglutide treatment had no effect on the fasting and postprandial glucagon concentrations in our patient. Thus, a higher concentration of GLP-1 may have been necessary to suppress glucagon secretion in our patient.

Whether GLP-1 receptors occur in adipose tissue is still controversial [3]. Expression of the GLP-1 receptor in adipose tissue is absent or very low [17], whereas GLP-1 induces adiponectin mRNA expression in 3T3L1 adipocytes [18]. GLP-1 reduces body weight and plasma leptin concentration in obese mice [19]. In our patient, plasma leptin concentration decreased immediately after liraglutide treatment. Similarly, plasma adiponectin concentration decreased. Subsequently, her appetite remained suppressed and body weight decreased. Although the precise mechanism of how GLP-1 suppresses leptin secretion is still unknown, liraglutide is effective for reducing body weight and appetite.

In this case, administration of only 0.3-mg liraglutide was sufficient to improve plasma glucose and beta cell function. In Japanese patients with T2DM, 0.1, 0.3, 0.6, and 0.9 mg liraglutide dose-dependently reduced HbA1c versus placebo by 0.79, 1.22, 1.64 and 1.85%, respectively [20]. In Caucasians, 1.2 and 1.8 mg liragulutide was used. GLP-1 sensitivity in Japanese patients may be much better than in Caucasian patients.

In conclusion, we report a patient for whom liraglutide effectively improved glycemic control similar to IIT. Normalizing fasting hyperglycemia is beneficial for successful introduction of the GLP-1 analog liraglutide in patients with T2DM.

Conflict of interest The authors state that they have no conflict of interest.

References

1. Wajchenberg BL. Beta-cell failure in diabetes and preservation by clinical treatment. Endocr Rev. 2007;28:187–218.
2. Nauck MA. Incretin-based therapies for type 2 diabetes mellitus: properties, functions, and clinical implications. Am J Med. 2011;124:S3–18.
3. Holst JJ. The physiology of glucagon-like peptide 1. Physiol Rev. 2007;87:1409–39.
4. Wójcicki JM. Mathematical descriptions of the glucose control in diabetes therapy. Analysis of the Schlichtkrull "M"-value. Horm Metab Res. 1995;27:1–5.
5. Inoguchi T, Umeda F, Kakimoto M, Sako Y, Ishii H, Noda K, Kunisaki M, Imamura M, Yu HY, Etoh T, Yoshikawa H, Aoki T, Hashimoto T, Nawata H. Chronic sulfonylurea treatment and hyperglycemia aggravate disproportionately elevated plasma proinsulin levels in patients with type 2 diabetes. Endocr J. 2000;47:763–70.
6. Coates PA, Ollerton RL, Luzio SD, Ismail I, Owens DR. A glimpse of the 'natural history' of established type 2 (non-insulin dependent) diabetes mellitus from the spectrum of metabolic and hormonal responses to a mixed meal at the time of diagnosis. Diabetes Res Clin Pract. 1994;26:177–87.
7. Yoshioka N, Kuzuya T, Matsuda A, Iwamoto Y. Effects of dietary treatment on serum insulin and proinsulin response in newly diagnosed NIDDM. Diabetes. 1989;38:262–6.
8. Laedtke T, Kjems L, Pørksen N, Schmitz O, Veldhuis J, Kao PC, Butler PC. Overnight inhibition of insulin secretion restores pulsatility and proinsulin/insulin ratio in type 2 diabetes. Am J Physiol Endocrinol Metab. 2000;279:E520–8.
9. Lynn FC, Pamir N, Ng EH, McIntosh CH, Kieffer TJ, Pederson RA. Defective glucose-dependent insulinotropic polypeptide receptor expression in diabetic fatty Zucker rats. Diabetes. 2001;50:1004–11.
10. Xu G, Kaneto H, Laybutt DR, Duvivier-Kali VF, Trivedi N, Suzuma K, King GL, Weir GC, Bonner-Weir S. Downregulation of GLP-1 and GIP receptor expression by hyperglycemia: possible contribution to impaired incretin effects in diabetes. Diabetes. 2007;56:1551–8.
11. Højberg PV, Vilsbøll T, Rabøl R, Knop FK, Bache M, Krarup T, Holst JJ, Madsbad S. Four weeks of near-normalisation of blood glucose improves the insulin response to glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide in patients with type 2 diabetes. Diabetologia. 2009;52:199–207.
12. Højberg PV, Zander M, Vilsbøll T, Knop FK, Krarup T, Vølund A, Holst JJ, Madsbad S. Near normalisation of blood glucose improves the potentiating effect of GLP-1 on glucose-induced insulin secretion in patients with type 2 diabetes. Diabetologia. 2008;51:632–40.
13. Degn KB, Juhl CB, Sturis J, Jakobsen G, Brock B, Chandramouli V, Rungby J, Landau BR, Schmitz O. One week's treatment with the long-acting glucagon-like peptide 1 derivative liraglutide (NN2211) markedly improves 24-h glycemia and alpha- and beta-cell function and reduces endogenous glucose release in patients with type 2 diabetes. Diabetes. 2004;53:1187–94.
14. van der Wal PS, Draeger KE, van Iperen AM, Martini C, Aarsen M, Heine RJ. Beta cell response to oral glimepiride administration during and following a hyperglycaemic clamp in NIDDM patients. Diabet Med. 1997;14:556–63.
15. Rizza RA. Pathogenesis of fasting and postprandial hyperglycemia in type 2 diabetes: implications for therapy. Diabetes. 2010;59:2697–707.
16. Kielgast U, Holst JJ, Madsbad S. Antidiabetic actions of endogenous and exogenous GLP-1 in type 1 diabetic patients with and without residual β-cell function. Diabetes. 2011;60:1599–607.
17. Bullock BP, Heller RS, Habener JF. Tissue distribution of messenger ribonucleic acid encoding the rat glucagon-like peptide-1 receptor. Endocrinology. 1996;137:2968–78.
18. Kim Chung le T, Hosaka T, Yoshida M, Harada N, Sakaue H, Sakai T, Nakaya Y. Exendin-4, a GLP-1 receptor agonist, directly induces adiponectin expression through protein kinase A pathway and prevents inflammatory adipokine expression. Biochem Biophys Res Commun. 2009;390:613–8.
19. He M, Su H, Gao W, Johansson SM, Liu Q, Wu X, Liao J, Young AA, Bartfai T, Wang MW. Reversal of obesity and insulin resistance by a non-peptidic glucagon-like peptide-1 receptor agonist in diet-induced obese mice. PLoS One. 2010;5:e14205.
20. Seino Y, Rasmussen MF, Zdravkovic M, Kaku K. Dose-dependent improvement in glycemia with once-daily liraglutide without hypoglycemia or weight gain: a double-blind, randomized, controlled trial in Japanese patients with type 2 diabetes. Diabetes Res Clin Pract. 2008;81:161–8.

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最新臨床糖尿病学 上

—糖尿病学の最新動向—

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その他の特定の機序・疾患による糖尿病

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Monogenic diabetes in children and young adults

堀川幸男

Key words：新生児糖尿病，遺伝子異常，インスリン分泌不全，MODY

はじめに

‘遺伝子異常による糖尿病’とは糖尿病の成因分類の‘III．その他の特定の機序，疾患によるもの’のうち，‘A．遺伝因子として遺伝子異常が同定されたもの’と，‘B-⑦ その他の遺伝的症候群で糖尿病を伴うことの多いもの’の一部が含まれる．糖尿病全体の数％を占めるにすぎないが，コモン2型糖尿病の病態解明のヒントを与える可能性もあり原因遺伝子の同定が進められてきた．原因遺伝子を同定することにより，その糖尿病の病型を正確に診断できるうえに，病態にあった治療法を選択することができ，予後の改善にもつながる．

本稿では，糖尿病を発症する単一遺伝子疾患について，肥満や脂肪萎縮を合併するものも含め解説する．

1 膵β細胞機能にかかわる遺伝子異常

1）インスリン分泌不全型を呈する遺伝子異常

新生児糖尿病(NDM)は生後6カ月未満に発症する糖尿病の総称で，高血糖やケトアシドーシスの発症を契機に発見され，インスリン治療を必要とすることが多い(**表1**)．約半数は生涯続く永続型(permanent neonatal diabetes mellitus: PNDM)であるが，残りの半数は主に生後1年以内に寛解する一過性型(transient neonatal diabetes mellitus: TNDM)である[1]．TNDMの40％は後に再発し，2型糖尿病の表現型を示す．臨床像からPNDMとTNDMを区別することは難しく，治療法の選択，予後の推測および遺伝カウンセリングにとって遺伝子検査は必須である．最初に述べる3つの遺伝子はNDMの原因として大きな割合を占めるものである．

a. INS遺伝子異常

糖尿病の発症年齢は新生児期から小児期，成人期にわたる．高IRI血症の場合は加齢など環境要因が加わり糖尿病が発症する型であるが，新生児発症型では遺伝子変異により，プロインスリンのミスフォールディングから小胞体ストレスがたまりβ細胞不全をきたしアポトーシスに至ると考えられている．多くは常染色体優性でPNDMの表現型を呈するが[2]，MODY(maturity-onset diabetes of the young)(MODY10)の報告もある．高IRI血症を示す症例だけでなく，MODY様あるいは自己抗体陰性で家族歴濃厚な1型糖尿病様の症例(type 1B)においては，インスリン遺伝子異常を疑いシークエンスを施行すべきである．日本人NDMにおけるインスリン遺伝子の詳細な頻度解析の報告はまだない．

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表1 新生児糖尿病の原因遺伝子リスト

遺伝子/染色体	PNDM/TNDM	遺伝形式	糖尿病以外の特徴
β細胞機能の低下			
KCNJ11/ABCC8	PNDM/TNDM	常優 or 常劣	発達遅延，てんかん
Chr.6q24	TNDM	様々	巨舌，臍帯ヘルニア
GCK	PNDM	常劣	両親が*GCK*ヘテロ変異による高血糖
SLC2A2	PNDM	常優	高ガラクトース血症，肝不全
GLIS3	PNDM	常劣	先天性甲状腺機能低下，緑内障，肝硬変，cystic kidney
NEUROD1	PNDM	常劣	小脳低形成，感音難聴，強度の近視，網膜低形成
PAX6	PNDM	常劣	小頭症，小眼症，汎下垂体機能低下症
膵低形成			
PTF1A	PNDM	常劣	膵臓および小脳の無形成
PDX1	PNDM	常劣	膵臓の無形成
HNF1B	PNDM/TNDM	常優	膵外分泌低下，腎嚢胞
NEUROG3	PNDM	常劣	先天性下痢，腸内分泌細胞欠失
RFX6	PNDM	常劣	小腸閉鎖，胆嚢低形成，下痢
β細胞破壊の増加			
EIF2AK3	PNDM	常劣	脊椎骨端の低形成，腎不全，繰り返す肝炎，精神発達遅延
FOXP3	PNDM	X染色体	免疫不全，難治性下痢，湿疹様皮疹，IgE高値
INS	PNDM	常優	なし
IER3IP1	PNDM	常劣	小脳症，重度小児てんかん性脳症

b. KCNJ11/ABCC8遺伝子異常

大部分の症例は孤発でその他は常染色体優性である．遺伝子異常の種類により，PNDMからMODY様のものまで様々な表現型をとるが，KCNJ11遺伝子異常ではPNDMが多く，ABCC8遺伝子異常ではTNDMが多い．スルホニルウレア薬(SU薬)が効を奏するが，必要量は通常の2型糖尿病で使用する場合よりも多い．20–25％にてんかん，発達遅延などの神経学的症候を合併するので(DEND syndrome; developmental delay, epilepsy, neonatal diabetes)，これに対するSU薬の効果についても検討が待たれる．コーカソイドの報告では，PNDMの原因の約半数はKATPチャネルを構成するKCNJ11(Kir6.2)とABCC8(SUR1)サブユニットの遺伝子異常である．残りのうち15–20％はINS遺伝子異常で他はまれである[3]．

c. 6q24領域の異常(TNDM1)

3種類の型の異常(父親由来のダイソミー，父親由来の6q24領域の重複，母親由来の第6番染色体のメチル化異常)があり，それによる父親由来のZAC/PLAGL1(zinc finger protein regulating apoptosis and cell cycle arrest; PACAPの1型受容体の転写調節因子)とHYMAI(hydatidiform mole associated and imprinted gene; untranslated mRNA)の過剰発現が原因である．また母親由来の第6番染色体のメチル化異常には，ZFP57遺伝子が常染色体劣性形式で関与しているといわれている．TNDMの大部分が6q24領域のインプリンティング異常に由来し，KCNJ11とABCC8の異常も一部存在する．日本人新生児糖尿病の遺伝子異常の報告でも，同様の傾向が認められている[4]．

d. MODY遺伝子異常

MODYの主たる病態は膵β細胞のインスリン分泌不全であるが，遺伝様式と発症年齢を除けば，MODYは‘やせ型インスリン分泌不全’を特徴とするコモン日本人2型糖尿病と類似した臨床像を呈する．そこで，その原因遺伝子が調べられた結果，現在までに6種類の主たる原因遺伝子(MODY1–6)が同定されている．なおMODY7–11は非常にまれと考えられている(表

表2 既知MODYの原因遺伝子と特徴

	遺伝子名	平均診断時年齢	特徴的臨床像	治 療
MODY1	*HNF4A*	17(5-18)	MODY3と類似 他に巨大児，低HDL血症，一過性新生児低血糖	少量SU薬
MODY2	*GCK*	10(0-18)	空腹時血糖値の軽微な上昇 妊娠糖尿病の5％	無治療
MODY3	*HNF1A*	14(4-18)	尿糖の閾値が低い→学校検尿で指摘されることあり 合併症の進行は1型や2型と同じ	SU薬によく反応
MODY4	*PDX1*	（3家系のみ）	ホモでPNDM，膵外分泌不全，膵の無形成．ヘテロではIGT～DMまで様々	食事療法～インスリン
MODY5	*HNF1B*	21(0-73)	腎嚢胞，腎異形成，性腺形成異常，膵低形成，肝機能異常，高尿酸血症など	食事療法～インスリン
MODY6	*NEUROD1*	（5家系のみ）	発症年齢や糖尿の重症度，合併症の進行などの表現型は様々	食事療法～インスリン
MODY7	*KLF11*	（3家系のみ）	酸化ストレスに対するβ細胞の感受性亢進？ *PDX1*の発現を調節？	無治療～インスリン
MODY8	*CEL*	（2家系のみ）	膵外分泌機能低下，軽度の腹痛，軟便，膵臓の線維化，萎縮	経口薬 インスリン
MODY9	*PAX4*	（2家系のみ）	軽症～進行した腎症まであり	食事療法 経口薬
MODY10	*INS*	11週(0-23歳)	新生児糖尿病では頻度が高いが，MODYとしてはまれ． 自己抗体陰性の1型糖尿病でみられることがある	インスリン
MODY11	*BLK*	（1家系のみ）	肥満が発症を修飾	食事療法～インスリン

2)．MODY1-6のうち，MODY2以外は転写因子の異常である．我が国ではMODY3が最も多く，その他のMODYはまれとされてきたが，著者らのスクリーニングでは，MODY2はそれほどまれではないことが判明している（未発表データ）．MODY2は一般に軽症であり空腹時の軽い高血糖や耐糖能異常のみで，糖尿病を発症するのは50％以下である．しかし，今後，グルコキナーゼ活性化薬の導入も予想され，本遺伝子変異の検索は，薬剤応答性を判断するうえで重要である．MODY3ではSU薬が効果的であり，多くの症例でインスリンからの切り替えが可能である．MODY5（RCAD: renal cysts and diabetes syndrome）ではHNF1β遺伝子の大規模遺伝子欠損による場合が40％と高率に認められ，直接シークエンスを第一選択としては見落とすので注意が必要である．また進行性の腎機能障害，腎嚢胞，腎低形成や奇形などを伴い，糖尿病の発症よりも先に認識されることも多い．なお原因遺伝子が不明のMODY（MODY-X）は，我が国では80％，欧米では20-30％を占めており，新規原因遺伝子同定が待たれる[5]．

e. ミトコンドリア糖尿病

mtDNA変異により，糖尿病以外に感音性難聴，脳筋症および心筋症などが認められる．3243点変異は日本人糖尿病患者の1％に認められ最も高頻度であり，低身長，痩せ型，30歳代発症および90％に感音性難聴などの臨床像がみられる．インスリン分泌は進行性に低下し，糖尿病合併症も進行しやすく，血中乳酸値は高値を示す[6]．

2）インスリン作用の伝達機構にかかわる遺伝子異常

a. インスリン受容体遺伝子異常

インスリン受容体（INSR）異常症は，常染色体劣性型が多く，同じインスリン受容体異常でも表現型の違いにより3種類に分けられる．インスリン受容体異常症A型，Rabson-Mendenhall症候群，妖精症（leprechaunism）の順に重症

化する．妖精症では子宮内発育不全，鞍鼻，空腹時低血糖，皮下脂肪の減少，陰核肥大を認め1歳未満で死亡する．Rabson-Mendenhall症候群はより軽症であり，発達遅延や歯牙異常を認め，青年期までに死亡することが多い．インスリン受容体異常症A型は最も軽症で発症が成年期と遅い．大量のインスリン投与でもコントロール困難な症例に対し，インスリンと50％の類似性をもつIGF-Iの投与が行われる．肥満を伴わない表皮黒色腫，高インスリン血症，高アンドロゲン血症を共通して認める[7]．

2 その他の遺伝的症候群で糖尿病を伴うことの多いもの

1）インスリン分泌不全型を呈する遺伝的症候群

a. Wolfram症候群

糖尿病以外に視神経萎縮，難聴，尿崩症を伴い，DIDMOAD症候群(diabetes insipidus, diabetes mellitus, optic atrophy and deafness)とも呼ばれ，平均寿命は30歳とされる．常染色体劣性遺伝であり，90％以上がWFS遺伝子異常である．糖尿病治療は，発症時よりインスリンが必要である[8]．

b. Wolcott-Rallison症候群(WRS)

WRSはまれな常染色体劣性遺伝性疾患であり，多くは生後6カ月くらいに突然インスリン依存型糖尿病で発症する．低身長を主徴として，加齢とともに骨折，肝脾腫，腎障害，精神発達遅延，心血管異常などの症状が認められる．原因はタンパク翻訳調節因子のEIF2AK3であることが判明しており，膵β細胞のアポトーシスなどを介して糖尿病発症にかかわっていると考えられている[9]．

c. Roger症候群(thiamine-responsive megaloblastic anemia: TRMA症候群)

サイアミントランスポーターをコードするSLC19A2遺伝子異常による常染色体劣性遺伝形式をとるまれな疾患で，小児期発症の糖尿病，貧血，感音性難聴を特徴とする[10]．

d. IPEX症候群(immune dysregulation, polyendocrinopathy, enteropathy, X-linked)

FOXP3遺伝子異常による伴性劣性遺伝形式の疾患で，免疫調節異常・多発性内分泌障害・腸症などが認められる．変異を受け継いだ男子のみが，致死性の自己免疫性・炎症性・アレルギー性免疫疾患を発症する．膵臓，甲状腺，大腸，皮膚など様々な臓器に炎症・組織破壊が起こり，通常生後1，2年以内に死亡する[11]．

2）インスリン抵抗性型を呈する遺伝的症候群

a. 先天性脂肪過多疾患

a）Alstrom症候群

小児期からの肥満を特徴とする常染色体劣性疾患であり，インスリン抵抗性型糖尿病，脂質異常症，網膜色素変性および難聴などの知覚神経異常を呈する．一般に精神遅滞や性腺の発育遅延は認められない．原因遺伝子はALMS1遺伝子が同定されているが，ホルモン感受性調節にかかわっている可能性がいわれている[12]．

b）Bardet-Biedl症候群

小児期からの肥満を特徴とするが，特に内臓脂肪蓄積が顕著であり，高血圧と糖尿病以外に，精神遅滞と性腺の発育遅滞，四肢の形態異常，網膜変性および腎臓の形態・機能異常などがよく認められる．原因遺伝子座は少なくとも8カ所以上存在することが判明しており，BBS1，BBS2，BBS4の異常でほとんどの症例が説明される．機能はまだ不明な部分が多いが，神経細胞などの繊毛運動機能に関係するといわれている[13]．

c）Prader-Willi症候群

新生児期の筋緊張低下，精神発達遅滞，食欲亢進による肥満，視床下部性内分泌異常などが認められる疾患で，原因は第15番染色体15q11-13の刷り込み領域の異常が同定されている．原因遺伝子としてnecdinなどが考えられている[14]．

他にレプチンやレプチン受容体の遺伝子変異から著明な肥満を介して高率に糖尿病を発症するが，ここでは省略する．またDown症候群，

Turner症候群，Klinefelter症候群などの染色体異常，筋強直性ジストロフィーなども肥満あるいは筋肉減少からインスリン抵抗性を介して糖尿病を発症することが多い．

b．先天性脂肪萎縮症

インスリン抵抗性と重度の脂質異常症，脂肪肝を認める場合には，一度は考慮すべき疾患である．遺伝学的に不均一でまれな疾患で，部分的あるいは全身性の皮下脂肪組織を欠損する．

a）部分型脂肪萎縮症

常染色体優性の部分型脂肪萎縮症が最も多く，核膜成分を構成するlamin A/CをコードするLMNA遺伝子異常が原因とされている．生下時の皮下脂肪は正常であるが，思春期になると四肢と体幹の皮下脂肪萎縮が明瞭となる．いまだ機能的に不明な点も多いが，LMNA遺伝子が筋・脂肪細胞のアポトーシスに関与している可能性がいわれている[15]．

b）全身性脂肪萎縮症

常染色体劣性の全身性脂肪萎縮症（Berardinelli-Seip症候群）のうち，BSCL1（AGPAT2）とBSCL2（Seipin）異常が95％以上を占めるが，臨床的な区別は困難である．AGPAT2の低下によりIL-6やTNF-αの発現が脂肪組織で亢進することが報告されている[15]．Seipinはリン脂質の代謝に関係しているとの報告があるが，まだ不明な点も多い．肝腫大が多く，先端巨大症様の顔貌がみられることもあり，糖尿病は20歳までに発症することが多い．

その他，PPARγの優性阻害型変異や老化症候群であるWerner症候群でも脂肪萎縮からインスリン抵抗性の亢進が認められているが，詳細はここでは省略する．

3）どちらにも分けられないもの

a．Fanconi-Bickel症候群

常染色体劣性遺伝性疾患であり，肝臓および腎臓におけるグリコーゲンの過剰蓄積，近位尿細管障害，グルコースとガラクトースの利用障害が特徴的である．原因は糖輸送体であるGLUT2（SLC2A2）であることが判明しており，空腹時の低血糖と対照的に食後は，グルコース応答性インスリン分泌の低下と肝臓への糖の取り込みが阻害されるため，著明な高血糖が認められる．治療としてはGLUT5が輸送体であるフルクトースを単純糖質として摂取する[16]．

おわりに

本稿では主に単一遺伝子の配列異常で糖尿病発症が説明されている例を紹介したが，今後は，CNVなどの遺伝子の構造変異やエピジェネティクスを含めて原因遺伝子探索を進めねばならないことも疑いなく，これにより初めて糖尿病の遺伝素因の全貌を明らかにすることができると考える．

文　献

1) Greeley SA, et al: Neonatal diabetes: an expanding list of genes allows for improved diagnosis and treatment. Curr Diabetes Rep **11**: 519-532, 2011.
2) Edghill EL, et al: Insulin mutation screening in 1,044 patients with diabetes: mutations in the INS gene are a common cause of neonatal diabetes but a rare cause of diabetes diagnosed in childhood or adulthood. Diabetes **57**: 1034-1042, 2008.
3) Murphy R, et al: Clinical implications of a molecular genetic classification of monogenic β-cell diabetes. Nat Clin Prac Endocrinol Metab **4**: 200-213, 2008.
4) Suzuki S, et al: Molecular basis of neonatal diabates in Japanese patients. J Clin Endocrinol Metab **92**: 3979-3985, 2007.
5) Fajans SS, Bell GI: MODY: history, genetics, pathophysiology, and clinical decision making. Diabetes Care **34**: 1878-1884, 2011.
6) Suzuki S: Diabetes mellitus with mitochondrial gene mutations in Japan. Ann N Y Acad Sci **1011**: 185-192, 2004.
7) Longo N, et al: Genotype-phenotype correlation in inherited severe insulin resistance. Hum Mol Genet **11**: 1465-1475, 2002.

8) Rohayem J, et al: Diabetes and neurodegeneration in Wolfram syndrome: a multicenter study of phenotype and genotype. Diabetes Care **34**: 1503–1510, 2011.
9) Rubio-Cabezas O, et al: Wolcott-Rallison syndrome is the most common genetic cause of permanent neonatal diabetes in consanguineous families. J Clin Endocrinol Metab **94**: 4162–4170, 2009.
10) Bergmann AK, et al: Thiamine-responsive megaloblastic anemia: identification of novel compound heterozygotes and mutation update. J Pediatr **155**: 888–892.e1, 2009.
11) Rubio-Cabezas O, et al: Clinical heterogeneity in patients with FOXP3 mutations presenting with permanent neonatal diabetes. Diabetes Care **32**: 111–116, 2009.
12) Girard D, Petrovsky N: Alström syndrome: insights into the pathogenesis of metabolic disorders. Nat Rev Endocrinol **7**: 77–88, 2011.
13) Zaghloul NA, Katsanis N: Mechanistic insights into Bardet-Biedl syndrome, a model ciliopathy. J Clin Invest **119**: 428–437, 2009.
14) Aebischer J, et al: Necdin protects embryonic motoneurons from programmed cell death. PLoS One **6**: e23764, 2011.
15) Capeau J, et al: Human lipodystrophies: genetic and acquired diseases of adipose tissue. Endocr Dev **19**: 1–20, 2010.
16) Taha D, et al: Hyperglycemia and hypoinsulinemia in patients with Fanconi-Bickel syndrome. J Pediatr Endocrinol Metab **21**: 581–586, 2008.

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Associations of plasma von Willebrand factor ristocetin cofactor activity and 5-hydroxyindole acetic acid concentrations with blood flow in lower-leg arteries in Japanese type 2 diabetic patients with normal ankle-brachial index

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ABSTRACT

Aims: To evaluate the associations of circulating levels of proinflammatory molecules and endothelial factors with blood flow in lower-leg arteries in diabetic patients with normal ankle-brachial index (ABI>0.9).
Methods: We enrolled 123 type 2 diabetic patients with normal ABI and 30 age-matched nondiabetic subjects consecutively admitted to our hospital. Flow volume and resistive index, an index of peripheral vascular resistance, at the popliteal artery were evaluated using gated two-dimensional cine-mode phase-contrast magnetic resonance imaging. An automatic device was used to measure ABI and brachial-ankle pulse-wave velocity (baPWV) for evaluation of arterial stiffness. Plasma soluble intercellular adhesion molecule-1 (sICAM-1) and monocyte chemoattractant protein-1 (MCP-1) concentrations, serum high-sensitivity C-reactive protein (hsCRP) levels, plasma von Willebrand factor ristocetin cofactor activity (VWF), and plasma vasoconstrictor serotonin metabolite 5-hydroxyindole acetic acid (5-HIAA) concentrations were measured.
Results: Diabetic patients had higher baPWV ($P<.0001$), resistive index ($P<.0001$), sICAM-1 ($P<.0001$), MCP-1 ($P=.0224$), log hsCRP ($P<.0001$), VWF ($P<.0001$), 5-HIAA ($P=.0015$), and lower blood flow ($P<.0001$) than nondiabetic subjects. VWF ($P=.0019$) or 5-HIAA ($P=.0011$), but not sICAM-1, MCP-1, and log hsCRP, was negatively correlated with blood flow in diabetic patients. A multivariate analysis revealed that the significant independent determinants of blood flow were hypertension, use of renin–angiotensin system inhibitors, VWF and 5-HIAA ($r^2=0.198$, $P<.0001$) in diabetic patients.
Conclusions: Plasma VWF and 5-HIAA concentrations are associated with blood flow and are involved in the pathogenesis of impaired peripheral circulation due to higher arterial stiffness and greater vascular resistance in lower-leg arteries in diabetic patients with normal ABI.

1. Introduction

Lower-extremity arterial disease is a major cause of ischemic limb, foot ulcers, and leg amputation in diabetic patients (Faglia et al., 2009; Gorogawa et al., 2006). Diabetic patients are known to have two distinct types of insufficient arterial blood flow to the lower limbs associated with the vessel wall properties. The diabetic condition promotes atherosclerotic plaque formation in the vessel wall and leads to peripheral artery disease (PAD), resulting in reduced blood supply to lower limbs during exercise or at rest. To help identify high-risk patients with PAD, the ankle-brachial index (ABI) is generally used (American Diabetes Association, 2003). The diabetic condition also causes higher arterial rigidity and greater vascular resistance to blood flow, resulting in reduced blood supply in the lower-leg arteries even though the individual has a normal ABI (> 0.9) (Suzuki et al., 2001). It has been reported that waveform analysis at the popliteal artery provides a powerful tool for identifying impaired peripheral circulation caused by either occlusive arterial disease or increased arterial stiffness and peripheral vascular resistance in diabetic patients using gated two-dimensional cine-mode phase-contrast magnetic resonance imaging (2D-cine-PC MRI) (Suzuki et al. 2001). In Japanese patients with diabetes, elderly patients (>65 years) had a higher prevalence of PAD (12.7%) compared with younger patients (<65 years) (4.0%) (Maeda et al., 2008). Prevalence of diabetic patients with low ABI (<0.90) and intermittent claudication is similar to that of diabetic patients with normal ABI and reduced blood flow in lower-leg arteries, indicating that increase in arterial stiffness and vascular resistance to blood flow may be one of the major causes of lower-extremity arterial disease in Japan (Suzuki et al., 2003).

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Endothelial cells actively regulate vascular tone and permeability, the balance between coagulation and fibrolysis and interaction with platelets (Davignon & Ganz, 2004). Vascular endothelium is resistant to interaction with circulating platelets under normal circumstances. An adhesive glycoprotein, von Willebrand factor (VWF), is synthesized by endothelial cells and stored in intracellular granules. When endothelial cells are injured, this molecule mediates platelet adhesion to the inflamed endothelial cells and participates in thrombus formation to arrest hemorrhage at the sites of vascular injury (Ruggeri & Mendolicchio, 2007). Serotonin is a monoamine neurotransmitter mainly synthesized in the enterochromaffin cells of the gastrointestinal mucosa and is released into the portal blood. This molecule is either rapidly stored in platelets for use in vasoconstriction to stop bleeding or metabolized by the liver and kidney to 5-hydroxyindole acetic acid (5-HIAA) (Tyce, 1990). Associations of these endothelial factors with peripheral circulation in lower-leg arteries among diabetic patients with normal ABI are not fully understood.

In the present study, we attempted to clarify whether circulating levels of VWF or 5-HIAA are associated with blood flow in lower-leg arteries in type 2 diabetic patients with normal ABI using gated 2D-cine-PC MRI.

2. Patients and methods

2.1. Patients

One hundred twenty-three type 2 diabetic patients and 30 nondiabetic subjects ranging in age from 45 to 75 years consecutively admitted to our hospital between May 2006 and March 2009 were recruited for the study. All diabetic patients were admitted for strict glycemic control or assessment of diabetic complications including eye, renal, neurological, and circulatory disorders. Diabetic patients taking antiplatelet agents for the primary prevention of cardiovascular disease and diabetic patients with clinical history of cerebrovascular disease, coronary artery disease, or PAD were excluded from the study. Patients who had abused alcohol or had foot edema caused by heart failure, liver cirrhosis, severe nephropathy (serum creatinine $>177\,\mu mol/l$), malignant neoplasm, autoimmune disorder, acute illness, or urinary tract infections were excluded from the study. Hydroxymethylglutaryl coenzyme A reductase inhibitors (statins) were used for the treatment of hyperlipidemia (LDL cholesterol ≥ 3.35 mmol/l) in diabetic patients. All diabetic patients with hypertension (>140/90 mm Hg) received renin–angiotensin system (RAS) inhibitors such as angiotensin-converting enzyme inhibitor (ACEI) or angiotensin II receptor blocker (ARB) for the management of high blood pressure. A 75-g glucose tolerance test was performed in our outpatient clinic for the diagnosis of patients with normal glucose regulation, impaired glucose tolerance, and diabetes mellitus (Alberti & Zimmet, 1998). Individuals with normal glucose tolerance were used as nondiabetic subjects in this study. The study was approved by the ethics committee of our institution, and informed consent was obtained from all patients before the examinations done during their stay in our hospital.

2.2. Clinical methods

Blood samples were drawn before breakfast in the morning after a 12-hour overnight fast. The HbA1c (%) is estimated as an NGSP equivalent value (%) calculated by the formula HbA1c (%) = HbA1c (JDS; %) + 0.4%, considering the relational expression of HbA1c (JDS; %) measured by the previous Japanese standard materials and measurement methods and HbA1c (NGSP) (NGSP [%] = 1.019 × JDS [%] + 0.30) and the coefficient of variance of 2%–3% in the measurement of HbA1c (The Committee of the Japan Diabetes Society on the Diagnostic Criteria of Diabetes Mellitus, 2010). Blood pressure was measured by a sphygmomanometer with the patients in the sitting position after 5 min of rest. Three readings separated by 2 min were taken, and the average was used for analysis. An automatic device (BP-203RPE; Colin, Komaki, Japan) was used to measure both ABI and brachial-ankle pulse-wave velocity (baPWV) as an index of arterial stiffness. A trained ophthalmologist carried out fundus ophthalmoscopies and classified diabetic patients as without retinopathy or as having simple, preproliferative, or proliferative retinopathy. Diabetic patients were classified by the measurement of urinary albumin excretion in 24-h urine collection as having normo-, micro-, or macroalbuminuria when at least two of three specimens were at diagnostic threshold of less than 30, 30–300, or greater than 300 mg/24 h, respectively. Estimated glomerular filtration rate (eGFR) was calculated by the modification of diet in renal disease formula with Japanese ethnic factor of 0.881 as follows: eGFR (ml/min per 1.73 m^2) $= 0.881 \times 186.3 \times Age^{-0.203} \times SCr^{-1.154}$ (if female $\times 0.742$), where SCr is serum creatinine (mg/dl) (Imai et al., 2007). Diabetic patients were screened for distal symmetric polyneuropathy using a 128-Hz tuning fork applied to the bony prominence at the dorsal surface of both great toes, just proximal to the nail bed. If the patient feels vibration for more than 10 s, vibration perception was regarded as a normal response (Boulton et al., 2005). Each subject was also classified based on smoking habits as being a current smoker or nonsmoker. Nonsmokers were defined as not having consumed tobacco for at least the previous 3 years. Plasma soluble intercellular adhesion molecule-1 (sICAM-1) and monocyte chemoattractant protein-1 (MCP-1) concentrations were measured by enzyme-linked immunosorbent assay kit (Human sICAM/CD54 or Human MCP-1 Quantikine ELISA kit; R&D Systems, Minneapolis, MN). Serum high-sensitivity C-reactive protein (hsCRP) levels were measured by a microparticle-enhanced immunonephelometric assay (CardioPhase hsCRP; Dade Behring, Newark, DE). Plasma von Willebrand factor ristocetin cofactor activity (VWF) was tested using reagents (BC von Willebrand Reagent; Dade Behring, Marburg, Germany). Plasma 5-HIAA concentrations were measured by the high-performance liquid chromatography system using a Model L-7100 pump (Hitachi, Tokyo, Japan) and a Model ECD-300 electrochemical detector (Eicom, Kyoto, Japan). An MRI scanner operating at 1.5-Tesla (Signa Horizon-LX; GE Medical Systems, Milwaukee, WI) was used for the following experimental protocols as previously described (Suzuki et al., 2001). All patients were at rest in the supine position during examinations, which were done in a temperature-controlled room at 25°C. A single slice at the popliteal artery was oriented perpendicular to the flow direction, and flow data were obtained using two-dimensional cine-mode phase-contrast magnetic resonance imaging with 80-cm/s velocity encoding triggered by peripheral gating. The accuracy and reproducibility of this methodology to measure flow volume for triphasic waveforms created from a pulsatile pump have been reported (McCauley et al., 1995). Flow data were analyzed on an Advantage Windows version 4.2 workstation (GE Medical Systems). The instantaneous flow volume at 20 equally spaced time points through the cardiac cycle was calculated from the individual velocity images by integrating the velocity across the area of the vessel. A resistive index, which is associated with arterial resistance to blood flow, has been defined as $(A-B)/A$, where A is the systolic peak velocity and B is the end-diastolic velocity (Halpern et al., 1998).

2.3. Statistical analysis

Statistical evaluation was done on SPSS software version 11.0 for Windows (SPSS Inc., Chicago, IL). Normality of distribution of each variable was assessed with the Kolmogorov–Smirnov test. Comparison between the two groups was performed using the unpaired Student's t-test. A multiple comparison of significant differences

among the three groups was carried out by one-way ANOVA followed by Scheffe's F test. The χ^2 test for 2-by-2 contingency table or Bonferroni test for 2-by-3 contingency table was used to compare frequencies between two or among three groups. Simple linear regression analyses were performed to assess the relation between normally distributed variables. Stepwise multiple regression analyses were performed to evaluate the association of blood flow with possible risk factors. The F value was set at 4.0 at each step. Values were expressed as the means ± SD. P values <.05 were considered statistically significant.

3. Results

3.1. Clinical characteristics in all subjects

Clinical characteristics in all subjects are shown in Table 1. There were no significant differences between the groups for prevalence of male gender, age, body mass index (BMI), LDL cholesterol (LDL-C), triglycerides (TGs), diastolic blood pressure (dBP), prevalence of smokers, and eGFR. However, diabetic patients had higher fasting plasma glucose (FPG) (P<.0001), hemoglobin A1c (HbA1c) (P<.0001), systolic blood pressure (sBP) (P=.0069), and frequency of micro- or macroalbuminuria (P<.0001) and neuropathy (P<.0001) and lower HDL cholesterol (HDL-C) (P=.0175) than nondiabetic subjects. Although ABI, heart rate, and early diastolic flow reversal were similar between the groups, diabetic patients had higher baPWV (P<.0001) and resistive index (P<.0001) and lower total (P<.0001), systolic (P=.0013), and late diastolic (P<.0001) flow volumes than nondiabetic subjects. Circulating levels of sICAM-1 (P<.0001), MCP-1 (P=.0224), log hsCRP (P<.0001), VWF (P<.0001), and 5-HIAA (P=.0015) in diabetic patients were higher than nondiabetic subjects.

Table 1
Clinical characteristics in diabetic patients with normal ABI and nondiabetic subjects.

	Nondiabetic subjects	Diabetic patients	P value
Number	30	123	–
Male gender (%)	15 (50.0)	78 (63.4)	.2534
Age (years)	60.3 ± 4.8	62.9 ± 7.8	.0828
BMI (kg/m^2)	22.8 ± 2.4	24.3 ± 4.2	.0594
Duration of diabetes (years)	–	10.8 ± 8.7	–
Treatment (diet/OHA/insulin)	–	5/67/51	–
FPG (mmol/l)	5.48 ± 0.59	7.85 ± 1.98	<.0001
HbA1c (%)	5.7 ± 0.4	8.9 ± 1.9	<.0001
LDL-C (mmol/l)	3.34 ± 0.75	3.07 ± 0.82	.0976
HDL-C (mmol/l)	1.54 ± 0.46	1.36 ± 0.36	.0175
TGs (mmol/l)	1.41 ± 0.58	1.60 ± 0.89	.2840
Statins (%)	–	26 (21.1)	–
Blood pressure (mm Hg)			
Systolic	125 ± 12	133 ± 15	.0069
Diastolic	79 ± 10	75 ± 10	.0698
ACEI or ARB (%)	–	41 (33.3)	–
Smokers (%)	8 (26.7)	35 (28.5)	.9999
Retinopathy (%)	–	48 (39.0)	–
Micro- or macroalbuminuria (%)	0 (0)	54 (43.9)	<.0001
eGFR (ml/min per 1.73 m^2)	71.5 ± 7.4	68.3 ± 15.9	.2906
Neuropathy (%)	0 (0)	80 (65.0)	<.0001
ABI	1.14 ± 0.07	1.13 ± 0.08	.6418
Brachial-ankle PWV (cm/s)	1407 ± 187	1673 ± 274	<.0001
Heart rate (bpm)	68 ± 10	68 ± 10	.8597
Flow volume (ml/min)			
Total	99.4 ± 19.2	69.6 ± 24.5	<.0001
Systolic	96.4 ± 20.9	81.9 ± 21.9	.0013
Early diastolic	−18.3 ± 14.0	−18.0 ± 10.6	.9193
Late diastolic	21.2 ± 8.5	5.7 ± 11.4	<.0001
Resistive index	0.999 ± 0.030	1.045 ± 0.048	<.0001
sICAM-1 (ng/ml)	150 ± 42	209 ± 73	<.0001
MCP-1 (pg/ml)	248 ± 60	281 ± 72	.0224
Log hsCRP	2.21 ± 0.38	2.69 ± 0.52	<.0001
VWF (%)	36.5 ± 16.0	91.9 ± 56.5	<.0001
5-HIAA (ng/ml)	4.1 ± 1.1	5.4 ± 2.2	.0015

Data are expressed as n (%) or means ± SD. OHA, oral hypoglycemic agent.

3.2. Associations of circulating molecules with vascular parameters

To clarify the associations of proinflammatory molecules (sICAM-1, MCP-1 and hsCRP) and endothelial factors (VWF and 5-HIAA) with vascular parameters (total flow volume, resistive index, and baPWV) in diabetic patients, simple linear regression analyses were performed as shown in Table 2. VWF (P=.0019) and 5-HIAA (P=.0011), but not sICAM-1, MCP-1, or log hsCRP, negatively correlated with total flow volume. Only 5-HIAA positively correlated with resistive index (P=.0199) and baPWV (P=.0003). To clarify the influence of 5-HIAA on blood flow, diabetic patients were classified into tertiles according to their 5-HIAA levels. Arterial waveforms recorded at the popliteal artery in each subgroup are shown in Fig. 1. The lowest group had a normal triphasic waveform, which could clearly be separated into systolic, early diastolic flow reversal, and late diastolic forward flow (Fig. 1A). The highest group showed reduced forward flow and new flow reversal in late diastole (Fig. 1C). Clinical characteristics and quantitative assessments of peripheral circulation in each subgroup are shown in Table 3. There were no significant differences among the groups for frequency of male gender, FPG, HbA1c, LDL-C, HDL-C, TGs, sBP, dBP, prevalence of patients taking statins or RAS inhibitors, frequency of smokers or neuropathy, sICAM-1, MCP-1, and log hsCRP. However, the highest group had the highest age (P=.0117), duration of diabetes (P=.0239), frequency of retinopathy (P<.01), and micro- or macroalbuminuria (P<.01) and VWF (P=.0297) and the lowest BMI (P=.0113) and eGFR (P=.0008) among the groups. There were no significant differences among the groups for ABI, heart rate, and systolic, early, and late diastolic flow volumes, while the highest group had the highest baPWV (P=.0453) and resistive index (P=.0383) and the lowest total flow volume (P=.0150) among the groups.

3.3. Variables associated with impaired blood flow

Stepwise multiple linear regression analyses were performed to examine the associations of blood flow with 10 possible risk factors for atherosclerosis (age, duration of diabetes, FBS, HbA1c, sBP, dBP, LDL-C, HDL-C, TGs, and smoking habit), three for microangiopathy (retinopathy, micro- or macroalbuminuria and eGFR), and two for medications (statins and RAS inhibitors) as well as two for endothelial factors (VWF and 5-HIAA). The significant independent determinants of blood flow were sBP (β = −0.287, F=4.447), VWF (β = −0.084, F=5.228), 5-HIAA (β = −2.382, F=6.047), and use of RAS inhibitors (β=12.538, F=7.640) (r^2=0.198, P<.0001) in diabetic patients.

4. Discussion

Our multivariate analysis demonstrates that the significant independent determinants of insufficient blood flow caused by

Table 2
Simple linear regression analyses of proinflammatory molecules (sICAM-1, MCP-1, and hsCRP) and endothelial factors (VWF and 5-HIAA) with vascular parameters (total flow volume and resistive index at the popliteal artery and baPWV) in diabetic patients with normal ABI.

	Total flow volume	Resistive index	baPWV
sICAM-1	n.s.	n.s.	n.s.
MCP-1	n.s.	n.s.	r=0.180, P=.0459
Log hsCRP	n.s.	n.s.	r=0.197, P=.0289
VWF	r=−0.277, P=.0019	r=0.238, P=.0081	n.s.
5-HIAA	r=−0.291, P=.0011	r=0.210, P=.0199	r=0.320, P=.0003

n.s., not significant.

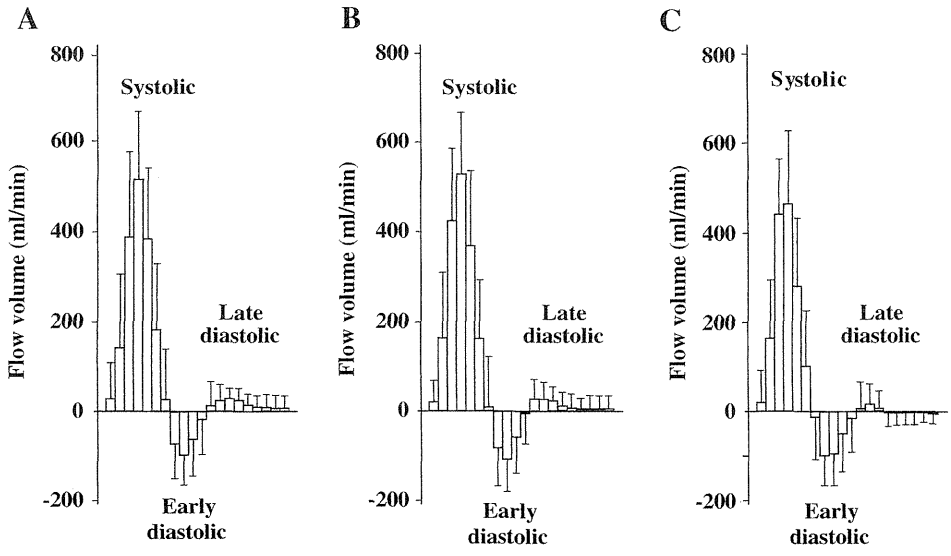

Fig. 1. Arterial waveforms recorded at the popliteal artery in diabetic patients with normal ABI grouped into tertiles according to their plasma 5-hydroxyindole acetic acid concentrations. (A) The lowest group. (B) The intermediate group. (C) The highest group. The instantaneous flow volume at 20 equally spaced time points through the cardiac cycle was reconstructed. Data are expressed as means ± SD.

higher arterial rigidity and greater peripheral vascular resistance in lower-leg arteries in diabetic patients with normal ABI are plasma levels of VWF and 5-HIAA, hypertension, and use of RAS inhibitors. An increase in plasma VWF concentration reflects endothelial damage and loss of adequate endothelial function. Plasma VWF concentration is elevated in diabetic patients with retinopathy (Morise et al., 1995) or nephropathy (Stehouwer et al., 1991). The release of serotonin from activated platelets is enhanced and intraplatelet serotonin content is diminished, resulting in elevated plasma serotonin concentrations in diabetic patients (Barradas et al., 1988; Malyszko et al., 1994). Plasma 5-HIAA concentrations are elevated and associated with arterial stiffness in diabetic patients (Fukui et al., 2007). Vasoconstrictor effects of 5-HIAA is extremely low compared to those of serotonin in normotensive rats (Thompson & Webb, 1987). Thus, the elevated plasma 5-HIAA concentrations found in this study might well contribute to higher arterial stiffness, greater vascular resistance, and lower blood flow in lower-leg arteries through vasoconstrictive actions of serotonin in diabetic patients with normal ABI.

Waveform analysis at the popliteal artery using gated 2D-cine-PC MRI is useful to assess peripheral circulation in both normal and diseased arteries (Suzuki et al., 2001). Normal subjects show a typically triphasic waveform, which can be clearly separated into systolic and early and late diastolic phases of the cardiac cycle. In late diastole, a positive waveform smaller than during systole occurs as the distended arterial reservoirs force blood antegrade through the arterioles into the venous circulation (Caputo & Higgings, 1992). When diabetic patients in this study were classified into tertiles based on their levels of 5-HIAA, those in the highest range demonstrate that waveform analysis at the popliteal artery is characterized not only by reduced forward flow but also a flow reversal in late diastole. Elastic arteries and muscular arteries have different vascular functions that are frequently impaired in diabetic patients (Henry et al. 2003; Kimoto et al., 2003). Large arteries, including the aorta and its major branches, exhibit elastic properties of the vessel wall and act as carrying vessels and blood supply reservoirs (London & Guerin, 1999). In the case of decreased arterial elasticity, less blood can be stored in these arteries, resulting in reduced late diastolic forward flow. The small-caliber arteries and arterioles act as resistance vessels that regulate blood flow to the capillaries (London & Guerin, 1999). Endothelial dysfunction and reduced limen diameter (Rizzoni et al., 2001a, 2001b) in small vessels are major determinants of peripheral vascular resistance. We have reported that diabetic patients with stiffer arteries show an abnormal vasculature in calf and foot arteries on magnetic resonance angiography (Suzuki et al., 2001), suggesting the presence of lumen narrowing or vessel wall distensibility even when ABI is normal. Angiographic evaluation in diabetic patients with foot ulcers reveals that stenosis involving ≥50% of the vessel lumen is found in 99% of the subjects and is detected in 16% of the subjects with normal ABI (Faglia et al., 1998). Vasodilatory capacity in common femoral artery is reduced in diabetic patients, with a more marked alteration in diabetic patients with microalbuminuria (Zenere et al., 1995). Capillary blood volume expansion during exercise assessed by near-infrared spectroscopy is impaired in the lower-extremity skeletal muscle of diabetic patients with normal ABI (Mohler et al., 2006). These findings support the notion that the presence of lumen narrowing or vessel wall distensibility and coexisting endothelial dysfunction in resistance vessels can contribute to hemodynamic change in diabetic patients even though they have a normal ABI.

Endothelium-dependent vascular relaxation is impaired in patients with essential hypertension (Panza et al., 1990). Nitric oxide is a potent vasodilator released by endothelial cells, and endothelial dysfunction attenuates vasodilation due to reduced nitric oxide bioavailability in diabetic patients (Williams et al., 1996). The use of RAS inhibitors ameliorates endothelial function in diabetic patients (Cheetham et al., 2000).

Among the limitations of this study, we used a cross-sectional study design in diabetic patients with normal ABI. Further prospective study is required to clarify the efficacy of oral administration of 5-HT2A receptor antagonist on plasma 5-HIAA concentrations and insufficient blood flow in lower-leg arteries in diabetic patients with normal ABI. In addition, our data were obtained in a Japanese population, and therefore, it remains to be established that these results can be generalized to other ethnicities.

In conclusion, we have revealed that plasma VWF and 5-HIAA concentrations are associated with blood flow, and these endothelial factors are involved in the pathogenesis of impaired peripheral

Table 3
Clinical characteristics of diabetic patients with normal ABI classified into tertiles based on the levels of plasma concentrations of 5-HIAA.

Group and range of 5-HIAA (ng/ml)	Lowest 1.7–4.3 (3.4 ± 0.6)	Intermediate 4.4–5.9 (5.0 ± 0.5)	Highest 6.0–13.6 (7.9 ± 1.9)
Number	41	41	41
Male gender (%)	24 (58.5)	28 (68.3)	26 (63.4)
Age (years)	60.4 ± 8.7	62.9 ± 7.4	65.5 ± 6.5[a]
BMI (kg/m^2)	25.6 ± 4.3	24.4 ± 4.3	22.8 ± 3.7[a]
Duration of diabetes (years)	7.6 ± 7.1	11.9 ± 8.2	12.8 ± 9.9[a]
Treatment (diet/OHA/insulin)	2/24/15	2/21/18	1/22/18
FPG (mmol/l)	7.69 ± 1.73	8.03 ± 2.07	7.82 ± 2.17
HbA1c (%)	8.9 ± 2.0	8.9 ± 2.0	8.7 ± 1.8
LDL-C (mmol/l)	3.22 ± 0.87	3.09 ± 0.77	2.90 ± 0.81
HDL-C (mmol/l)	1.29 ± 0.31	1.39 ± 0.40	1.39 ± 0.36
TGs (mmol/l)	1.63 ± 0.96	1.58 ± 0.87	1.58 ± 0.87
Statins (%)	7 (17.1)	10 (24.4)	9 (22.0)
Blood pressure (mm Hg)			
Systolic	130 ± 16	131 ± 14	138 ± 15[a]
Diastolic	76 ± 11	75 ± 9	74 ± 12
ACEI or ARB (%)	12 (29.3)	14 (34.1)	15 (36.6)
Smokers (%)	11 (26.8)	12 (29.3)	12 (29.3)
Retinopathy (%)	9 (22.0)	15 (36.6)	24 (58.5)[b]
Micro- or macroalbuminuria (%)	12 (29.3)	15 (36.6)	27 (65.9)[b,d]
eGFR (ml/min per 1.73 m^2)	73.1 ± 13.4	71.5 ± 16.1	60.3 ± 15.3[c,e]
Neuropathy (%)	23 (56.1)	29 (70.7)	28 (68.3)
ABI	1.13 ± 0.07	1.13 ± 0.09	1.14 ± 0.08
Brachial-ankle PWV (cm/s)	1604 ± 223	1660 ± 294	1754 ± 285[a]
Heart rate (bpm)	70 ± 11	67 ± 11	68 ± 8
Flow volume (ml/min)			
Total	73.9 ± 23.5	76.2 ± 23.3	58.6 ± 23.5[a,e]
Systolic	85.3 ± 23.4	85.6 ± 19.3	74.8 ± 21.6
Early diastolic	−18.5 ± 8.8	−17.2 ± 10.1	−18.4 ± 12.7
Late diastolic	7.1 ± 11.3	7.7 ± 11.2	2.2 ± 11.2
Resistive index	1.040 ± 0.048	1.035 ± 0.046	1.062 ± 0.046[e]
sICAM-1 (ng/ml)	203 ± 71	205 ± 78	217 ± 72
MCP-1 (pg/ml)	270 ± 64	283 ± 76	291 ± 77
Log hsCRP	2.66 ± 0.47	2.76 ± 0.60	2.66 ± 0.49
VWF (%)	76.1 ± 51.7	90.5 ± 54.3	109.0 ± 59.7[a]

Data are expressed as n (%) or means ± SD. OHA, oral hypoglycemic agent.
[a] $P<.05$ vs. the lowest group.
[b] $P<.01$ vs. the lowest group.
[c] $P<.001$ vs. the lowest group.
[d] $P<.05$ vs. the intermediate group.
[e] $P<.01$ vs. the intermediate group.

circulation in lower-leg arteries in diabetic patients with normal ABI. These findings contribute to understanding the mechanism of insufficient arterial blood flow to the lower limbs in diabetes.

References

Alberti, K. G., & Zimmet, P. Z. (1998). Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabetic Medicine, 15*(7), 539–553.

American Diabetes Association. (2003). Peripheral arterial disease in people with diabetes. *Diabetes Care, 26*(12), 3333–3341.

Barradas, M. A., Gill, D. S., et al. (1988). Intraplatelet serotonin in patients with diabetes mellitus and peripheral vascular disease. *European Journal of Clinical Investigation, 18*(4), 399–404.

Boulton, A. J., Vinik, A. I., et al. (2005). Diabetic neuropathies: a statement by the American Diabetes Association. *Diabetes Care, 28*(4), 956–962.

Caputo, G. R., & Higgings, C. B. (1992). Magnetic resonance angiography and measurement of blood flow in the peripheral vessels. *Investigative Radiology, 27*(Suppl 2), S97–S102.

Cheetham, C., Collis, J., et al. (2000). Losartan, an angiotensin type 1 receptor antagonist, improves endothelial function in non-insulin-dependent diabetes. *Journal of the American College of Cardiology, 36*(5), 1461–1466.

Davignon, J., & Ganz, P. (2004). Role of endothelial dysfunction in atherosclerosis. *Circulation, 109*(23 Suppl 1), III27–III32.

Faglia, E., Favales, F., et al. (1998). Angiographic evaluation of peripheral arterial occlusive disease and its role as a prognostic determinant for major amputation in diabetic subjects with foot ulcers. *Diabetes Care, 21*(4), 625–630.

Faglia, E., Clerici, G., et al. (2009). Long-term prognosis of diabetic patients with critical limb ischemia: a population-based cohort study. *Diabetes Care, 32*(5), 822–827.

Fukui, M., Ose, H., et al. (2007). Association between urinary albumin excretion and plasma 5-hydroxyindole-3-acetic acid concentration in men with type 2 diabetes. *Diabetes Care, 30*(10), 2649–2651.

Gorogawa, S., Iida, O., et al. (2006). Characteristics of peripheral arterial disease in Japanese type 2 diabetic patients. *Diabetologia, 49*(6), 1449–1451.

Halpern, E. J., Merton, D. A., et al. (1998). Effect of distal resistance on Doppler US flow patterns. *Radiology, 206*(3), 761–766.

Henry, R. M., Kostense, P. J., et al. (2003). Arterial stiffness increases with deteriorating glucose tolerance status: the Hoorn Study. *Circulation, 107*(16), 2089–2095.

Imai, E., Horio, M., et al. (2007). Estimation of glomerular filtration rate by the MDRD study equation modified for Japanese patients with chronic kidney disease. *Clinical and Experimental Nephrology, 11*(1), 41–50.

Kimoto, E., Shoji, T., et al. (2003). Preferential stiffening of central over peripheral arteries in type 2 diabetes. *Diabetes, 52*(2), 448–452.

London, G. M., & Guerin, A. P. (1999). Influence of arterial pulse and reflected waves on blood pressure and cardiac function. *American Heart Journal, 138*(3 Pt 2), 220–224.

Maeda, Y., Inoguchi, T., et al. (2008). High prevalence of peripheral arterial disease diagnosed by low ankle-brachial index in Japanese patients with diabetes: The Kyushu Prevention Study for Atherosclerosis. *Diabetes Research and Clinical Practice, 82*(3), 378–382.

Malyszko, J., Urano, T., et al. (1994). Daily variations of platelet aggregation in relation to blood and plasma serotonin in diabetes. *Thrombosis Research, 75*(5), 569–576.

McCauley, T. R., Peña, C. S., et al. (1995). Validation of volume flow measurements with cine phase-contrast MR imaging for peripheral arterial waveforms. *Journal of Magnetic Resonance Imaging, 5*(6), 663–668.

Mohler, E. R., III, Lech, G., et al. (2006). Impaired exercise-induced blood volume in type 2 diabetes with or without peripheral arterial disease measured by continuous-wave near-infrared spectroscopy. *Diabetes Care, 29*(8), 1856–1859.

Morise, T., Takeuchi, Y., et al. (1995). Increased plasma levels of immunoreactive endothelin and von Willebrand factor in NIDDM patients. *Diabetes Care, 18*(1), 87–89.

Panza, J. A., Quyyumi, A. A., et al. (1990). Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *New England Journal of Medicine, 323*(1), 22–27.

Rizzoni, D., Porteri, E., et al. (2001). Endothelial dysfunction in small resistance arteries of patients with non-insulin-dependent diabetes mellitus. *Journal of Hypertension, 19*(5), 913–919.

Rizzoni, D., Porteri, E., et al. (2001). Structural alterations in subcutaneous small arteries of normotensive and hypertensive patients with non-insulin-dependent diabetes mellitus. *Circulation, 103*(9), 1238–1244.

Ruggeri, Z. M., & Mendolicchio, G. L. (2007). Adhesion mechanisms in platelet function. *Circulation Research, 100*(12), 1673–1685.

Stehouwer, C. D., Stroes, E. S., et al. (1991). von Willebrand factor and development of diabetic nephropathy in IDDM. *Diabetes, 40*(8), 971–976.

Suzuki, E., Egawa, K., et al. (2003). Prevalence and major risk factors of reduced flow volume in lower extremities with normal ankle-brachial index in Japanese patients with type 2 diabetes. *Diabetes Care, 26*(6), 1764–1769.

Suzuki, E., Kashiwagi, A., et al. (2001). Increased arterial wall stiffness limits flow volume in the lower extremities in type 2 diabetic patients. *Diabetes Care, 24*(12), 2107–2114.

The Committee of the Japan Diabetes Society on the Diagnostic Criteria of Diabetes Mellitus. (2010). Report of the committee on the classification and diagnostic criteria of diabetes mellitus. *Journal of Diabetes Investigation, 1*(5), 212–228.

Thompson, L. P., & Webb, R. C. (1987). Vascular responsiveness to serotonin metabolites in mineral corticoid hypertension. *Hypertension, 9*(3), 277–281.

Tyce, G. M. (1990). Origin and metabolism of serotonin. *Journal of Cardiovascular Pharmacology, 16*(Suppl 3), S1–S7.

Williams, S. B., Cusco, J. A., et al. (1996). Impaired nitric oxide-mediated vasodilation in patients with non-insulin-dependent diabetes mellitus. *Journal of the American College of Cardiology, 27*(3), 567–574.

Zenere, B. M., Arcaro, G., et al. (1995). Noninvasive detection of functional alterations of the arterial wall in IDDM patients with and without microalbuminuria. *Diabetes Care, 18*(7), 975–982.

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Effectiveness of the glucagon test in estimating islet function for liraglutide treatment in a lean diabetic patient with impaired insulin response to glucose

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CASE REPORT

Effectiveness of the glucagon test in estimating islet function for liraglutide treatment in a lean diabetic patient with impaired insulin response to glucose

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Abstract A 52-year-old lean woman was admitted to hospital for a medical examination in July 2010. A 75 g oral glucose test (75 g OGTT) revealed postprandial hyperglycemia (above 200 mg/dl at 120 min) and she was diagnosed with diabetes mellitus. She was negative for diabetes-related autoantibodies. The 75 g OGTT also revealed a very low insulinogenic index (I.I.) of 0.024 μU/ml/mg/dl; however, her plasma C-peptide immunoreactivity (CPR) response to glucagon was preserved (0.94 and 5.56 at 0 and 6 min, respectively). At the same time, she also suffered from Fe-deficiency anemia due to endometriosis, for which treatment with leuprorelin was initiated after hospitalization in August 2010. Meanwhile, her postprandial plasma glucose level continued to increase. Subsequently, on November 30, 2010, alogliptin therapy at a dose of 6.25 mg was initiated, which was increased to 25 mg on December 28, 2010. Leuprorelin therapy was discontinued on January 14, 2011; however, her plasma glucose level remained high despite the alogliptin therapy. Administration of 0.3 mg liraglutide was initiated on March 15, 2011, and the dose was increased to 0.6 mg on June 7, 2011. The plasma glucose level, glycosylated hemoglobin (HbA1c) level, and 1.5 anhydroglucitol level gradually improved, as did her I.I. Moreover, fasting plasma glucagon levels were suppressed by liraglutide. Her fasting serum CPR to plasma glucose ratio and homeostasis model assessment (HOMA-R) were low both before and after liraglutide administration. These results suggest that the fasting CPR to glucose ratio may underestimate residual islet function in lean patients with high insulin sensitivity. Thus, a glucagon test may be useful to estimate residual islet function when administering liraglutide treatment in lean diabetes patients.

Keywords Liraglutide · Glucagon-like peptide · Glucagon test · Glucose responsiveness

Introduction

Type 2 diabetes mellitus (T2DM) is emerging as a major social and economic problem across the world. This form of diabetes is characterized by a combination of insulin resistance and β-cell dysfunction [1]. Unlike Caucasian T2DM, Japanese T2DM is characterized by impaired insulin response to glucose rather than insulin resistance [2]. To overcome impaired glucose-stimulated insulin secretion, several medications such as glinides, biguanides, α-glucosidase inhibitors, thiazolidine, and rapid-acting insulin are widely used. However, these drugs have severe side effects, including nausea, abdominal bloating, indigestion, hypoglycemia, and weight gain. Recently, glucagon-like peptide-1 (GLP-1) analogs have emerged as promising drugs with significantly less severe side effects [3].

GLP-1 and glucose-dependent insulinotropic peptide (GIP) are gastrointestinal hormones that comprise the incretins [4]. GLP-1 and GIP are secreted in the small intestine from L cells and K cells, respectively [4]. GLP-1

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is responsible for potentiating insulin secretion, delaying gastric emptying, lowering appetite and reducing body weight, and suppressing glucagon secretion [4]. In contrast, GIP lacks all of these actions except for the potentiation of insulin secretion [4]. GLP-1 analogs are therefore commonly used to maintain glucose homeostasis without the side effects of body weight gain and hypoglycemia [3–5].

In T2DM patients generally, the impaired insulin response to glucose is caused by abnormal glucose sensing, a decreased number of pancreatic islets, and/or abnormal accumulation of toxic molecules such as amylin [6–8]. Some groups have reported that pancreatic islet volumes are much lower in T2DM patients than in healthy subjects [6].

Thus, evaluation of residual β-cell function is important to determine whether insulin treatment is warranted [9–12]. Fasting serum C-peptide immunoreactivity (CPR) to plasma glucose ratio and glucagon tests are often used to estimate pancreatic islet function in order to determine the requirement for insulin therapy [11, 12]. However, clinical estimation of the amount of residual islet function, which is required for the successful introduction of a GLP-1 analog, remains elusive.

We report the case of a 52-year-old lean diabetic patient whose islet function was estimated using the fasting CPR to plasma glucose ratio and a glucagon test to determine the feasibility of liraglutide treatment. The results show that the glucagon test can be beneficial for the introduction of liraglutide therapy in a lean diabetic patient with impaired insulin response to glucose.

Case report

A 52-year-old lean woman was admitted to hospital for a medical examination in July 2010. On admission, her plasma glucose level was 203 mg/dl. A 75 g oral glucose tolerance test (75 g OGTT) was performed on July 12, 2010. Plasma glucose levels at 0, 30, 60, and 120 min were 100, 236, 228, and 238 mg/dl, respectively. On the basis of these findings, the patient was diagnosed with diabetes mellitus. Her insulinogenic index (I.I.), which reflects insulin response to glucose, was as low as 0.024 and she was hospitalized to undergo treatment for diabetes mellitus on August 9, 2010. She reported a family history of diabetes (two brothers) and an unremarkable past medical history except for hypermenorrhea and endometriosis.

Physical data on admission

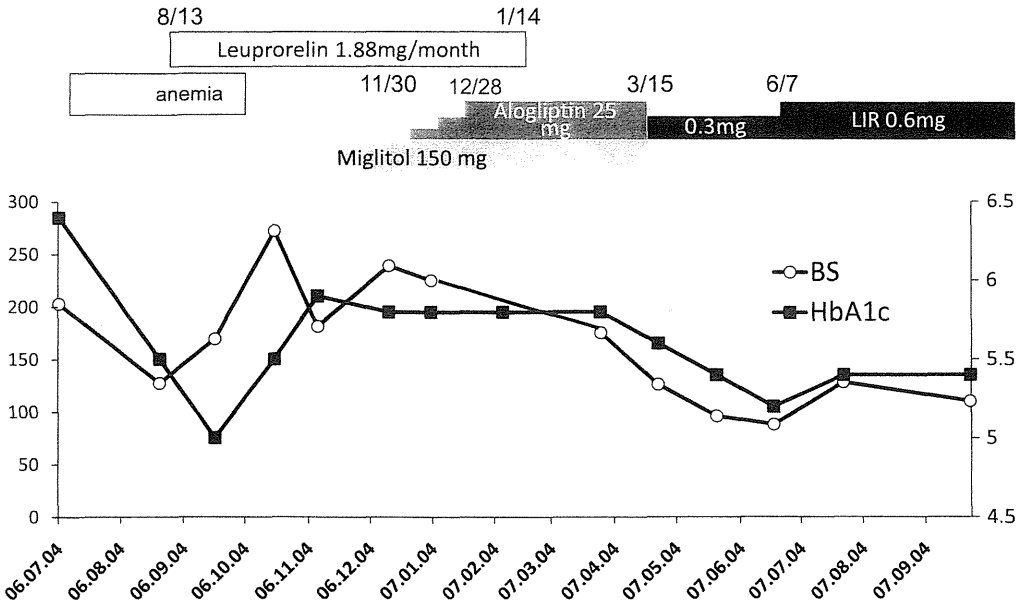
The patient's height and weight were 148.8 cm and 40.1 kg, respectively, with a body mass index of 18.11 kg/m^2. Her resting blood pressure was 92/63 mmHg. There were no abnormal findings in the thoracoabdominal region and no abnormal neurogenic signs were detected.

Laboratory findings

The laboratory data are presented in Table S1 of the Electronic supplementary material (ESM). Urinalysis revealed no abnormalities. Hemoglobin, iron, and ferritin levels were decreased, whereas unbound iron binding capacity was increased. She was diagnosed with iron-deficiency anemia caused by hypermenorrhea and endometriosis. Levels of transaminases, creatinine, electrolytes, blood urea nitrogen, and uric acid were within normal limits. The percentage value of glycosylated hemoglobin (HbA1c) was estimated as a National Glycohemoglobin Standardization Program (NGSP) equivalent value calculated by the formula HbA1c (%) = HbA1c (Japan Diabetes Society, JDS) (%) + 0.4%, considering the relational expression of HbA1c (JDS)(%) measured by the previous Japanese standard substance and measurement methods and HbA1c (NGSP) [13]. The HbA1c level was as high as 6.3% (NGSP equivalent value) [13]. Results for diabetes-related autoantibodies, including anti-glutamic acid decarboxylase antibody, anti-insulin antibody, and anti-IA2 antibody, were all negative. Unlike the insulin response to glucose in 75 g OGTT, the patient's plasma CPR response to glucagon on August 11, 2010 was preserved (0.94 and 5.56 ng/ml at 0 and 6 min, respectively). Her plasma insulin response to glucagon was similarly preserved (1.5 and 47.4 μU/ml). Her plasma glucose levels were measured at the same time (99 and 137 mg/dl at 0 and 6 min, respectively). Her CPR index was low (0.83 and 0.95 on August 10 and 11, 2010, respectively) (Fig. 1; Table S1).

Clinical course

Diet therapy (1200 kcal/day) and 150 mg miglitol were initiated after hospitalization on August 9, 2010. On August 10, 2010, the patient's plasma glucose levels at 0800, 1000, 1200, 1400, 1800, 2000, 2400, 0300, and 0800 hours were 98, 206, 144, 198, 109, 197, 112, 103, and 98 mg/dl, respectively. The corresponding insulin levels at 0800 and 1000 hours were 0.81 and 4.36 ng/ml, respectively. Administration of leuprorelin (1.88 mg/month) for endometriosis was initiated on August 13, 2010. During the course of leuprorelin treatment, the patient's plasma glucose level (1400 hours) gradually increased. Her HbA1c levels increased after an initial decrease because of the restoration of anemia. To improve postprandial hyperglycemia, she was administered 6.25 mg of alogliptin + 150 mg of miglitol on November 30, 2010. The dose of



	2010.8.10	2011.2.9	2011.5.25	2011.9.7
FPG (mg/dl)	98	111	106	103
CPR (ng/ml)	0.81	0.98	0.74	0.68
CPR index	0.83	0.88	0.7	0.66
proinsulin (pmol/l)	<2	<2	4.5	2.3
Insulinogenic Index	ND	0.034	0.08	0.044
glucagon (pg/ml)	67	51	22	29
HOMA-R	0.267	0.6	0.55	0.25
1,5-AG (μg/ml)	ND	15	17.1	17.5

Fig. 1 Clinical course of the patient. Miglitol at a dose of 150 mg was initiated on August 9, 2011. After the administration of leuprorelin (1.88 mg/month) was initiated, her plasma glucose level increased immediately. On November 30, 2010, 6.25 mg of alogliptin was initiated; this dose was increased to 25 mg on December 25, 2010. On March 15, 2011, administration of 0.3 mg liraglutide was initiated; this dose was increased to 0.6 mg on June 7, 2011, and continued thereafter. After the administration of liraglutide, plasma glucose levels (1400 hours) (*open circles*) and HbA1c (*filled squares*) gradually decreased. Fasting plasma glucose, C-peptide, proinsulin, glucagon, and 1,5-anhydroglucitol (1,5-AG) levels were measured on August 10, 2010, February 9, 2011, May 25, 2011, and September 7, 2011. CPR index was calculated by the formula CPR index = CPR (ng/ml)/glucose (mg/dl) × 100. Insulinogenic index (I.I.) was calculated by the formula I.I. = (insulin at 30 min − insulin at 0 min)/(glucose at 30 min − glucose at 0 min). HOMA-R was calculated by the formula HOMA-R = fasting glucose (mg/dl) × insulin (μU/ml)/405

alogliptin was increased to 25 mg on December 28, 2010. On January 14, 2011, the course of leuprorelin therapy was completed. To evaluate the effect of alogliptin on glucose tolerance, we performed a 75 g OGTT on February 9, 2011. Plasma glucose levels at 30, 60, and 120 min were much higher than those measured on July 12, 2010, although her insulin levels were also higher (Fig. 2). This indicated worsening glycemic control. To protect the pancreatic β cells from hyperglycemia, we recommended intensive insulin therapy with a low dose of rapid-acting insulin three times per day. However, the patient rejected our proposal because she was afraid of hypoglycemia. As an alternative, we recommended GLP-1 analog therapy, which she accepted. Administration of 0.3 mg liraglutide began on March 15, 2011. To evaluate the effect of liraglutide on blood glucose control, we performed a 75 g OGTT on May 25, 2011. Plasma glucose levels at 30, 60, and 120 min were improved compared with those measured on July 12, 2010 and February 9, 2011. The dose of liraglutide was increased to 0.6 mg on June 7, 2011. After the introduction of liraglutide, her plasma glucose and HbA1c levels immediately improved (Fig. 1). A 75 g OGTT performed on September 7, 2011 revealed that her plasma glucose levels at 30, 60, and 120 min were below 200 mg/dl (Fig. 2). When the dose of liraglutide was increased to 0.6 mg, she experienced soft stools and indigestion; nevertheless, her body weight remained unchanged throughout the course of liraglutide treatment.

To evaluate the effect of treatment with the various medications used, we performed 75 g OGTT on July 12, 2010 (no medication); February 9, 2011 (25 mg alogliptin + 150 mg miglitol); May 25, 2011 (0.3 mg liraglutide); and September 7, 2011 (0.6 mg liraglutide). The areas under the curve of glucose (AUCglucose) in the 75 g

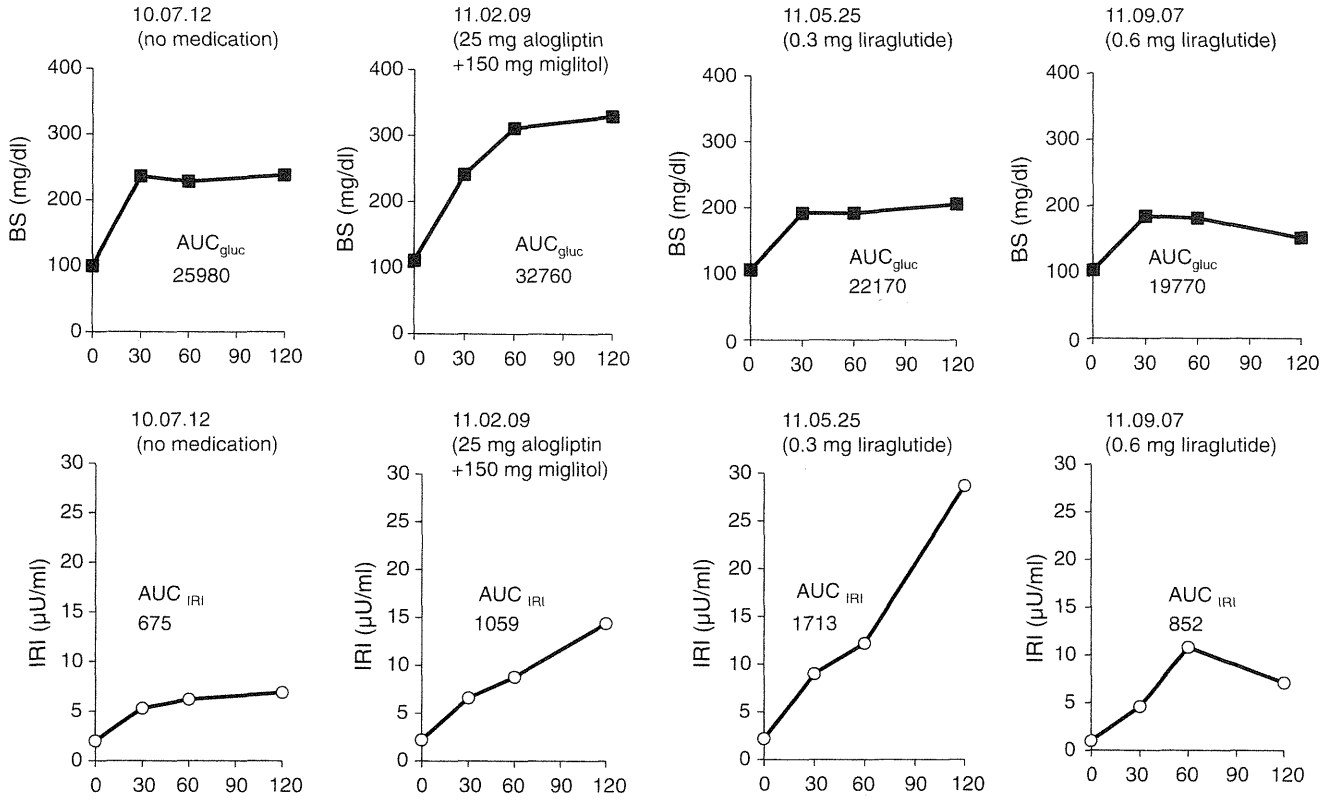


Fig. 2 The effects of plasma glucose and insulin levels on the 75 g oral glucose tolerance test for the various medications used in this patient. We performed a 75 g oral glucose tolerance test (75 g OGTT) on July 12, 2010, February 9, 2011, May 25, 2011, and September 7, 2011. Plasma glucose (*filled squares*) and plasma insulin (*open circles*) levels were measured at 0, 30, 60, and 120 min

OGTTs conducted on the abovementioned dates were 25980, 32760, 22170, and 19770 mg/dl min, respectively. Consistent with this result, HbA1c (6.4, 5.8, 5.4, and 5.4%, respectively) and 1.5-anhydroglucitol (1,5-AG) levels (15, 17.1, and 17.5 μg/ml in February, May, and September 2011, respectively) also showed an improving trend, particularly after liraglutide administration. The area under the curve of insulin (AUCinsulin) improved from 675 μU/ml min and 1059 μU/ml min in July 2010 and February 2011, respectively, to 1713 and 852 μU/ml min in May and September 2011 (after liraglutide administration), respectively. Similarly, the I.I. improved from 0.024 and 0.034 μU/ml/mg/dl in July 2010 and February 2011, respectively, to 0.080 and 0.044 μU/ml/mg/dl in May and September 2011 (after liraglutide administration), respectively (Fig. 2). Plasma proinsulin levels improved from <2.0 and <2.0 pmol/l in July 2010 and February 2011, respectively, to 4.5 and 2.3 pmol/l in May and September 2011 (after liraglutide administration), respectively (Fig. 1), whereas fasting plasma glucagon levels decreased from 67 and 51 pg/ml in July 2010 and February 2011, respectively, to 22 and 29 pg/ml in May and September 2011 (after liraglutide administration), respectively (Fig. 1).

Discussion

We report that the glucagon test was beneficial for assessing islet function when administering liraglutide treatment in a lean diabetic patient with impaired insulin response to glucose. Liraglutide improved glucose tolerance by delaying gastric emptying, suppressing the glucagon level, and improving insulin sensitivity. Thus, in cases where the insulin response to glucagon, but not to glucose, is preserved and insulin resistance is moderate, liraglutide may be an effective alternative for the treatment of postprandial hyperglycemia.

Glucagon, GLP-1, and GIP potentiate glucose-stimulated insulin secretion and insulin synthesis by increasing the intracellular cyclic adenosine monophosphate (cAMP) level [4]. In the present case, evidence of intact effects of cAMP on islet function were as follows: (1) insulin response to glucose was preserved, (2) postprandial serum CPR (4.36 ng/ml) and proinsulin levels (23.2 pmol/l) were much higher than fasting CPR (0.81 ng/ml) and proinsulin (<2 pmol/l) levels, and (3) I.I., which reflects first-phase insulin release, was not improved by liraglutide, which potentiated only second-phase insulin secretion. These results suggest that the effect of cAMP on insulin granule