厚生労働科学研究費

循環器疾患等生活習慣病対策総合研究事業

循環器リスクと耐糖能障害の効率的な健診マーカーの探索

平成２０年度　総括研究報告書

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【１】総括研究報告

厚生労働省科学研究費補助金（循環器疾患等生活習慣病対策総合研究事業）
総括研究報告書

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研究要旨

２型糖尿病は軽症段階や予備軍であっても心血管イベントの重要リスクとなることが明らかとなった。しかし、耐糖能障害も循環器疾患は共に独立した不均一な病態であり、遺伝的背景も含めて人種差も存在する。しかしながら、日本人においては、両者に共通した固有の病態や機序の理解は充分とは言いがたい。このような状況では効率的な発症予防を行なうには、画一的でなく、日本人の病態とリンクした健診項目の設定と個々の体質や生活特性に配慮した保健指導が必要である。初年度は日本人の病態の調査を行い、インスリン分泌不全と肥満・抵抗性の評価を試みた。

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. 研究目的

圣症糖尿病や境界型（予備軍）は心血管イベント
)高リスクである。しかし、耐糖能障害も循環器
ミ患も共に不均一な病態なので、効率的な予防を
亍なうには、日本人の病態とリンクした健診項目
)設定と個々の体質や生活特性に配慮した保健指導が必要である。

平成15-17年度に、脳梗塞多発地域の恵那保健所
管区で、糖尿病型と境界型住民162人(>HbA1c 5.5
を対象として保健師による生活指導介入を行な
た（６ヶ月間）。その結果、大半に病型改善が
められ、そのプライミング効果は１年後も継続
た。そこで同研究を拡大し、感受性素因と不応
因を特定して健診項目と指導内容に反映させる
とを考案した。

本研究では、明確に判別された耐糖能に従って
糖尿病型、境界型、正常型の３群を設定し、糖
質代謝を亜分類する生化学検査、動脈硬化に関
る血液検査、健康・生活に関する質問票調査を
施し、保健指導のフォローアップ解析を行なう。
即ち、２年後に75g OGTTと血液検査の再検と生
習慣調査を再施行し、耐糖能の変化と関連する
中因子と生活因子を特定する。さらに、日本人
心筋梗塞と有意に関連する約30種類の遺伝子多

ーカー(N Engl J Med. 347:1916-23, 2002)と関
因子の関連解析を行ない、感受性体質に基づい
テイラーメイド指導を可能とする健診マーカー
開発する。

. 研究方法

(糖脂質代謝異常の病型分類)

ての検査は本研究が提携して登録した医療機関(岐阜市内)にて実施した。被検者1,070人について糖負荷の前値と60分値インスリン(IRI)を測し、各々HOMA-R(抵抗性指数)、HOMA-β(インリン分泌量)、insulinogenic index(分泌指数)算出して病型を亜分類した。

HOMA-R = 空腹時血糖(mg/dl) X 空腹時インスリン値(μU/ml)/405

HOMA-β = 空腹時インスリン値(μU/ml) X 360/空腹時血糖(mg/dl)ー63

Insulinogenic index = ΔIRI/ΔBS

ンスリン感受性や120分血糖値は各々が単独で心血管イベントに関連するので、日本人の病型の質的区分は重要である。血中脂質(TG, HDL, DL)も同時測定し、脂質異常症の有無の検討と病分類をする。肥満度(BMI)と内蔵脂肪の蓄積度ウエスト周囲径、体脂肪率、V(内蔵)/S(皮下))を評価する。

(動脈硬化の血液検査)

脈硬化の質的な背景は、インスリン抵抗性、炎に起因、血管の石灰化、それ以外の4群に分類きることを既に明らかにしている。各々に関係る血中の液性因子を測定する(レジスチン、TNF-、アディポネクチン、hsCRP、IL6など)。120血糖の高値は血栓形成と関連するので血中PAI-1も測定する。蓄積脂肪の量的変化と心血管イベントに関連する独自開発の因子も測定し、格や生活因子との関連を解析する。

別集団(糖尿病患者を主体とする)では、上の液性因子の測定に加えて、画像診断の導入により動脈硬化の定量的評価を試みている(冠動脈石灰化度、内頸動脈のIMT測定、ABI、PWV、内脂肪の定量など)。腎機能の評価(eGFRの算定)も同時に実施した。

(生活状況調査)

自記式質問票調査(36ページ)を作成した(添資料)。本質問票により、性別、生年月日、家環境、職業等の背景、既往歴、20歳時からの体の増減、健康状態、睡眠時間、喫煙習慣と受動煙、歯の衛生、家族の人数と既往歴、糖尿病の識(24問)、ストレステスト(12問)、食習慣と事内容、健康食品、嗜好品、運動習慣、QOL-26、女性の妊娠・出産歴・閉経などの情報収集を行った。

<u>倫理面への配慮</u>

全ての実験はヘルシンキ宣言と3省庁合同指を遵守して行われる。本計画は医学部の遺伝子析と臨床研究に関する倫理審査委員会の承認をに受けている。患者および健常者からのDNAと液試料はインフォームドコンセントを取得した後に提供を受け、連結可能匿名化の状態で保存される。臨床上の個人情報を含めて、研究ソースはべて本研究に関わらない秘守義務を負う識別管者(研究機関が指定)が管理する。保存コンピュータはインターネットに連結せず、専用で独立ある。

C. 結果

糖尿病型、境界型、正常型に区分した結果、

尿病型 9.3% (男性 12.9%、女性 6.9%) 、境界型 22.6% (男性 23.9%、女性 22.6%) であり、HbA1c による全国調査の集計結果と同程度であった。一方、耐糖能異常に肥満が占める割合は41.2%であった。糖尿病型の出現頻度に男女差が認められたが、平均体重の差異に基づく可能性が示唆された (肥満：男性 50.9%、女性 37.2%) 。一方、境界型においても同様の体重の男女差が認められたにも関わらず (男性 46.6%、女性 30.7%) 、境界型の出現頻度は男女間で同程度であったので、肥満と関連が少ない日本人の罹患体質が示唆された。

HOMA-Rとinsuulinogenic indexを算定した結果、インスリン抵抗性は境界型 (男2.0±2.0、女1.5±1.9) と糖尿病型 (男21±1、女2.8±2.7) では共にボーダー領域であり、発症への直接関与は少ないと考えられた。一方、インスリン分泌については、正常型から境界型 (男0.5±0.4、女0.6±0.4) 、糖尿病型 (男0.2±0.1、女0.2±0.4) に移行するにつれ初期分泌が低くなり、欧米のような代償性の高分泌は認められなかった。興味深いことに、insuulinogenic indexは、肥満も非肥満も数値も減弱傾向も同パターンを呈したので、日本人においては、インスリン分泌不全が一義的な病態と結論された。インスリン分泌量の指標であるHOMA-βにおいても、欧米で見られるような境界型での代償性の高インスリン分泌は認められず、糖尿病型に至って減少が肥満と非肥満の両者で同様に認められた。

D. 考察

日本人の40歳以上における耐糖能異常は、全体の約30%に認められた。肥満 (BMI >25) の合併によって、糖尿病や境界型の頻度は上昇をみたが、全体における肥満の割合は約40%であり、非肥満が過半数を占めた。このことは、肥満や内臓脂肪 (ウエスト周囲径) を必須の検討項目とする特定健診 (いわゆるメタボ健診) では日本人固有の体質に基づく2型糖尿病の発症を見逃す公算が大であることを示唆する。

罹患体質は生活要因に大きく修飾される。本研究では、多項目に亘る生活要因を半定量化できるように設定して質問票として調査した。次年度はこれらの情報と耐糖能型や指導後変化との関連を解析することによって感受性素因を検出できることを期待している。また、遺伝子多型の解析を導入することによって、関連解析は罹患素因と生活習慣指導の感受性を提示するであろう。現在、すべての項目でータを入力中である。

E. 結論

日本人では糖尿病や心血管病の発症には分泌不全を基盤として、軽度のインスリン抵抗性が関与する図式が考えられた。耐糖能異常に肥満が占める割合は約40%であり、肥満と腹囲を必須項目としたメタボ健診では、重要リスクである糖尿病を高率に見逃す危険性が考えられ、見直しが必要と考えられた。

F 健康危険情報

なし

G 研究発表

論文発表 (原著論文)

T. Yoshimura, E. Suzuki, I. Ito, M. Sakaguchi T. Uzu, Y. Nishio, H. Maegawa, S. Morikawa, T Inubushi, A. Hisatomi, K. Fujimoto, J. Takeda A. Kashiwagi.
Impaired peripheral circulation in lower-leg arteries caused by higher arterial stiffness and greater vascular resistance associated with nephropathy in type 2 diabetic patients with normal ankle-brachial indices.

iabetes Res Clin Prac. 80: 416-423, 2008.

. Hirota, E. Suzuki, I. Ito, M. Ishiyama, S.
oto, Y. Horikawa, T. Asano, M Kanematsu, H.
oshi, J. Takeda.
oronary artery calcification, large artery
tiffness and renal insufficiency associate
ith serum levels of tumor necrosis factor-a in
apanese type 2 diabetic patients.
iabetes Res Clin Prac. 82: 58-65, 2008.

. Suzuki, T. Yoshimura, Y, Omura, M. Sakaguchi,
. Nishio, H. Maegawa, A. Hisatomi, K. Fujimoto,
. Takeda, A. Kashiwagi.
igher arterial stiffness, great peripheral
ascular resistance and lower blood flow in
ower-leg arteries are associated with
ong-term hyperglycaemia in type 2 diabetic
atients with normal ankle-brachial index.
iabetes Metab Res Rev. (In press)

. Oba, C. Nagata, K. Nakamura, N. Takatsuka,
. Shimizu.
elf-reported diabetes mellitus and risk of
ortality from all causes, cardiovascular
isease, and cancer in Takayama: a
opulation-based prospective cohort study in
apan.
Epidemiol. 18: 197-203, 2008.

. Oba, K. Nakamura, Y. Sahashi, A. Hattori, C.
agata.
onsumption of vegetables alters morning
rinary 6-sulfatoxymelatonin concentration.
Pineal Res. 45: 17-23, 2008.

K. Nakamura, C. Nagata, S. Oba, N. Takatsuka
H. Shimizu.
Fruit and vegetable intake and mortality frc
cardiovascular disease are inversel
associated in Japanese women but not in men.
J Nutr. 138: 1129-1134, 2008.

山本眞由美、川手靖彦、戸谷理恵子、武田　純、
梅本敬夫、紀ノ定保臣
岐阜県医師会病診連携システムにおける、糖尿
病診連携サポートシステムの試作
肥満と糖尿病　7: 56-61, 2008.

山本眞由美、塩谷真由美、堀川幸男、武田　純
岐阜市における糖尿病診療の実態調査
岐阜県医師会雑誌　21: 51-56, 2008.

岡安伸二、下田浩欣、紀ノ定保臣、武田純、山
眞由美
インスリンの安全管理に関する電子カルテ機能
有用性と問題点
肥満と糖尿病 7 : 28-35, 2008.

下田浩欣、岡安伸二、紀ノ定保臣、武田純、山
眞由美
電子カルテ情報から分析する大学病院の糖尿病
棟患者の特徴分析の試行～業務を可視化する有
性について
肥満と糖尿病 7 : 89-93, 2008.

山本眞由美、武田純、紀ノ定保臣
糖尿病の遠隔病診連携を支援する岐阜県医師会
診連携システム構築の報告
日本遠隔医療学会雑誌 4, 325-327, 2008.

学会発表

(国内)

高見和久、武田則之、山田明子、勝田　純、猿井
こ、川地真一、佐々木昭彦、武田　純
ピオグリタゾンとメトフォルミンの比較：ピオグ
リタゾンが有効であった２型糖尿病患者の特性
第５１回日本糖尿病学会年次学術集会、５月、東
京、2008

岡安伸二、下田浩欣、伊藤善規、紀ノ定保臣、武
田純、山本眞由美
インスリンの安全管理に関する電子カルテ機能の
有用性と問題点
第５１回日本糖尿病学会年次学術集会、５月、東
京、2008

下田浩欣、高塚直能、紀ノ定保臣、岡安伸二、伊
藤善規、武田純、山本眞由美
電子カルテ情報マイニングシステムを用いて分析
した糖尿病専門病棟患者の動向について
第５１回日本糖尿病学会年次学術集会、５月、東
京、2008

村瀬　寛、鈴木英司、勝田　純、則竹伸保、早川
和硯、高見和久、山田明子、廣田卓男、伊東　勇、
武田　純
磁気共鳴装置を用いた糖尿病患者における下肢機
能性血流障害と血小板機能異常の解明
第５１回日本糖尿病学会年次学術集会、５月、東
京、2008

山本眞由美
糖尿病の遠隔病診連携を支援する岐阜県医師会病
診連携システム構築の報告
日本遠隔医療学会 JTTA 2008 in Gifu、岐阜
2008. 10. 11.

山本眞由美、細江香苗、久保田芳則、武田純
糖尿病予備群に対する栄養指導成果の検討～岐阜
県恵那地域糖尿病協議会の取り組みから
第 12 回日本病態栄養学会年次学術集会、京都
2009. 1. 10-11.

田中生雅、佐渡忠洋、武田純、清水克時、山本眞
由美
肥満大学生の成果に影響する食行動についての検
討
第 12 回日本病態栄養学会年次学術集会、京都
2009. 1. 10-11.

(国際)

M. Yamamoto, Y. Kawade, R. Totani, J. Takeda
Y. Kinosada.
An electronic support system for diabetic
medicine to promote physician cooperation in
Gifu prefectural medical association.
The 8th Japan-China Friendship Symposium on
Diabetes Mellitus, Wakayama, 2008. 4. 25 -26.

S. Okayasu, Y. Kinosada, H. Shimoda, Y. Ito, J
Takeda, M. Yamamoto.
Effect of electronic patient medical record
(EPMR) system and computerized clinical
guideline on safety management of insulin
therapy.
The 8th Japan-China Friendship Symposium on
Diabetes Mellitus, Wakayama, 2008. 4. 25 -26.

M. Yamamoto, Y. Kawade, R. Totani, J. Totani
Y. Kinosada.
Electronic Support System for Diabetes
Management and Promotion of Physician
Cooperation in Gifu Prefecture, Japan.

he 68th ADA Scientific Sessions, San Francisco,

008.6.6-10.

知的財産権の出願・登録

1　特許取得

なし

2　実用新案登録

なし

3　その他

なし

【２】研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表雑誌名	巻	ページ	出版年
T. Yoshimura, et al.	Impaired peripheral circulation in lower-leg arteries caused by higher arterial stiffness and greater vascular resistance associates with nephropathy in type 2 diabetic patients with normal ankle-brachial indices.	Diabetes Research and Clinical Practice	80	416-423	2008
T. Hirota, et al.	Coronary artery calcification, large artery stiffness and renal insufficiency associate with serum levels of tumor necrosis factor-a in Japanese type 2 diabetic patients.	Diabetes Research and Clinical Practice	82	58-65	2008
S. Oba, et al.	Consumption of vegetables alters morning urinary 6-sulfatoxymelatonin concentration.	Journal of Pineal Research.	45	17-23	2008
S. Oba, et al.	Rationality/anti-emotionality personality and dietary habits in a community	Journal of Epidemiology	18	183-190	2008

	population in Japan. gene arc in prefrontal cortex of rats.				
K. Nakamura, et al.	Fruit and vegetable intake and mortality from cardiovascular disease are inversely associated in Japanese women but not in men.	Journal of Nutrition	138	1129-1134	2008
E. Suzuki, et al.	Higher arterial stiffness, great peripheral vascular resistance and lower blood flow in lower-leg arteries are associated with long-term hyperglycaemia in type 2 diabetic patients with normal ankle-brachial index.	Diabetes, Metabolism and Research Review		In press.	2009
S. Oba, et al.	Self-reported diabetes mellitus and risk of mortality from all causes, cardiovasucular disease and cancer in Takayama: A population-based prospective cohort study in Japan.	Journal of Epidemiology	18	197-203	2008
山本眞由美、他	岐阜県医師会病診連携システムにおける、糖尿病病診連携サポートシステムの試作	肥満と糖尿病	7	56-61	2008

山本眞由美、他	岐阜市における糖尿病診療の実態調査	岐阜県医師会雑誌	21	51-56	2008

【３】研究成果の刊行物・別刷

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Impaired peripheral circulation in lower-leg arteries caused by higher arterial stiffness and greater vascular resistance associates with nephropathy in type 2 diabetic patients with normal ankle–brachial indices

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Nephropathy

ABSTRACT

Diabetic nephropathy is a major cause of lower-limb amputation. We enrolled 250 type 2 diabetic patients without apparent occlusive peripheral arterial disease (ankle–brachial indices >0.9) and 40 age-matched nondiabetic subjects consecutively admitted to our hospital. Flow volume and resistive index (RI), an index of vascular resistance, at the popliteal artery were evaluated using gated two-dimensional cine-mode phase-contrast magnetic resonance imaging. Brachial–ankle pulse wave velocity (baPWV) was measured as an index of arterial distensibility. Flow volume was negatively correlated with both baPWV ($p = 0.0009$) and RI ($p < 0.0001$) among the patients. When the patients were grouped into four subgroups with or without albuminuria and renal insufficiency according to the levels of urinary albumin excretion rate (≥20 or <20 μg/min) and estimated glomerular filtration rate (eGFR) (<60 or ≥60 ml/min/1.73 m^2), albuminuric patients with renal insufficiency ($n = 30$) showed the lowest flow volume ($p = 0.0078$) and the highest baPWV ($p = 0.0006$) and RI ($p = 0.0274$) among the groups. Simple linear regression analyses demonstrated that eGFR correlated positively with flow volume ($p = 0.0020$) and negatively with baPWV ($p = 0.0258$) and RI ($p = 0.0029$) in patients with albuminuria ($n = 92$), but not with normoalbuminuria ($n = 158$). Impaired peripheral circulation in lower-leg arteries associates with nephropathy in diabetic patients even though they have normal ankle–brachial indices.

1. Introduction

Diabetic nephropathy is the leading cause of lower-limb amputation [1], end-stage renal failure and death from cardiovascular disease (CVD) [2]. Elevated urinary albumin excretion [3] and decrease in glomerular filtration rate [4] are powerful markers of increased cardiovascular morbidity and mortality for diabetic patients. Therefore, current diabetes guidelines recommend screening for elevated urinary albumin excretion as the earliest clinical evidence of nephropathy [5] in

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addition to screening for decline in estimated glomerular filtration rate (eGFR) calculated by the Modification of Diet in Renal Disease (MDRD) formula to detect chronic kidney disease attributed to diabetes [6]. Duplex Doppler sonography has demonstrated that alterations in glomerular capillaries and tubulointerstitial compartments of the kidney result in increase of intrarenal vascular resistance [7]. Type 2 diabetic patients with nephropathy are known to have elevated intrarenal vascular resistance compared with other types of renal disease [8]. Furthermore, among diabetic patients with nephropathy, increased vascular resistance in the intrarenal arteries associates with the severity of systemic atherosclerosis assessed by brachial–ankle pulse wave velocity (baPWV) as a marker of large artery stiffness [8], or intima-media thickness (IMT) in the carotid or femoral artery [9]. In the lower-leg arteries, diabetic patients show two types of insufficient arterial blood flow associated with the vessel wall properties. Firstly, diabetic condition is likely to increase atherosclerotic plaque formation in the vessel wall and promote peripheral artery occlusive disease (PAOD) in the lower extremities, resulting in reduced blood flow to lower limbs during exercise or at rest [10]. To help identify high-risk patients with PAOD, the ankle–brachial index (ABI) is generally used [11]. Secondly, arterial distensibility and vascular resistance reduce blood supply in the lower-leg arteries even though the individual has no apparent PAOD [12]. Gradual accumulation of advanced glycation end-products (AGEs) [13], increased IMT [14], and radiologically detectable calcified deposits in the vessel walls [15] are seen at different stages of the atherosclerotic process and are considered to be responsible for the pathogenesis of vascular rigidity. Endothelial dysfunction develops during the atherosclerotic process and is associated with reduction in vasodilator capacity and increase in peripheral vascular resistance [16]. Although it is desirable to ameliorate insufficient arterial blood flow before the onset of lower-limb ischemia, the role of diabetic nephropathy in peripheral circulation in lower-leg arteries has not been fully elucidated.

The aim of the present study was to clarify the association of impaired peripheral circulation in lower-leg arteries with nephropathy in type 2 diabetic patients with normal ABI by using a new technique of two-dimensional cine-mode phase contrast magnetic resonance imaging (2D-cine-PC MRI).

2. Materials and methods

We enrolled 250 type 2 diabetic patients and 40 age-matched nondiabetic subjects ranging in age from 50 to 75 years who had been consecutively admitted to our hospital. All patients were admitted for strict glycemic control or assessment of diabetic complications, and no patients had history of cerebrovascular disease, coronary arterial disease, and/or PAOD. Patients were considered to have cerebrovascular disease if they had a history of sudden focal neurological deficit. Coronary arterial disease was diagnosed if the patients had a history of myocardial infarction or showed abnormal electrocardiographic findings. PAOD was diagnosed if the patients had an abnormal ABI [11]. Patients who had foot edema caused by heart failure, liver cirrhosis or severe nephropathy (serum creatinine >2 mg/dl), malignant neoplasm, alcohol abuse, acute illness, urinary tract infections or hematuria were excluded from the study. Presence of pyuria or hematuria was diagnosed by microscopic examination and counting of the number of white blood cells or red blood cells per high-power field in the last voided urine of a 24-h collection. Every patient with hypertension (>140/90 mmHg) received antihypertensive treatment to reduce the risk of CVD events. Although administration of renin–angiotensin system inhibitors, including angiotensin converting enzyme inhibitor (ACEI) [17] and angiotensin II receptor blocker (ARB) [18], can decrease the urinary albumin excretion and slow the decline in glomerular filtration rate among type 2 diabetic patients, those medications were used for the management of raised blood pressure. The study was approved by the ethics committee of our institution, and informed consent was obtained from all patients before the examinations.

An automatic device (BP-203RPE; Colin, Komaki, Japan) was used to measure ABI and baPWV, as an index of the elastic properties of large arteries [19]. Because the PWV from the heart to the brachial artery in diabetic patients is similar to that in control subjects, baPWV is regarded as a quantitative measure of arterial stiffness from the heart to the ankle. A trained ophthalmologist carried out fundus ophthalmoscopies and identified diabetic patients as either without retinopathy, having simple retinopathy or having proliferative retinopathy. Patients with diabetes were screened for distal symmetric polyneuropathy using a 128-Hz tuning fork applied to the bony prominence at the dorsalis surface of both great toes, just proximal to the nail bed [20]. When the tuning fork was placed on the foot for 10 s, if the patients required >10 s to detect the vibration, vibration perception was regarded as compromised. Each patient was also identified by smoking habit as being a current smoker or non-smoker. Nonsmokers were defined as not having tobacco consumption for at least the previous 3 years. Urinary albumin excretion rate (AER) was measured in 24-h urine samples. Diabetic patients were classified as having normoalbuminuria ($n = 158$), microalbuminuria ($n = 56$), or overt proteinuria ($n = 36$) when the AER was <20, 20–200, or >200 μg/min, respectively. The Japanese ethnic factor for the MDRD equation has been reported to be 0.881 [21]. Therefore, the eGFR is calculated by the MDRD formula as follows: eGFR $(\text{ml/min}/1.73\ \text{m}^2) = 0.881 \times 186.3 \times \text{Age}^{-0.203} \times \text{SCr}^{-1.154}$ (if female $\times$ 0.742), where SCr is serum creatinine (mg/dl). With or without renal insufficiency was defined as eGFR of <60 or $\geq$60 ml/min/1.73 m^2 [22].

An MRI scanner operating at 1.5 T (Signa Horizon-LX; GE Medical Systems, Milwaukee, WI) was used for the following experimental protocols as previously described [23]. All patients were at rest in the supine position during examinations, which were done in a temperature-controlled room at 25 °C. To set up the individual flow analysis, the popliteal artery was depicted by gated 2D time-of-flight magnetic resonance angiography. A single slice was oriented perpendicular to the flow direction, and flow data were obtained using 2D-cine-PC MRI with 80-cm/s velocity encoding triggered by peripheral gating. Flow data were analyzed on an Advantage Windows version 4.2 workstation (GE Medical Systems, Milwaukee, WI) to determine the direction and velocity through the cardiac cycle. The instantaneous flow volume at 16 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 (RI), which allows quantitative analysis of the waveform and associates 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 [24].

Statistical evaluation was carried out on SPSS software version 11.0 for Windows (SPSS, Chicago, IL). Comparisons between the diabetic patients and their control group were done using the unpaired Student's t-test. A multiple comparison of significant differences among the four groups was carried out by one-way ANOVA followed by Scheffe's F-test. The chi-squared test for 2-by-2 or Bonferroni test for 2-by-4 contingency table was used to compare the frequencies between two or among four groups. Values were expressed as the means ± S.D. We considered p-values <0.05 to be statistically significant.

3. Results

3.1. *All subjects*

Clinical characteristics and vascular parameters in all subjects are shown in Table 1. There were no significant differences between the groups for prevalence of male sex, age, body mass index (BMI), total cholesterol (TC), prevalence of smoking status, and eGFR. However, compared with nondiabetic subjects, diabetic patients had higher fasting plasma glucose (FPG) ($p < 0.0001$), hemoglobin A1c (HbA1c) ($p < 0.0001$), triglycerides (TGs) ($p = 0.0261$), and systolic blood pressure (sBP) ($p = 0.0230$) and lower HDL cholesterol (HDL-C) ($p = 0.0024$), and diastolic blood pressure (dBP) ($p = 0.0202$). Although ABI, heart rate and systolic and early diastolic flow volumes were similar between the groups, diabetic patients had lower total ($p = 0.0005$) and late diastolic ($p < 0.0001$) flow volumes and higher baPWV ($p < 0.0001$) and RI ($p < 0.0001$) than those in the nondiabetic subjects, indicating that arterial stiffness and vascular resistance are possible risk factors for reduced blood flow in diabetic patients. To clarify the associations among those vascular parameters, simple linear regression analyses were performed. Total flow volume, baPWV, and RI were negatively (total flow volume vs. baPWV, $r = -0.209$, $p = 0.0009$; total flow volume vs. RI, $r = -0.645$, $p < 0.0001$) or positively (baPWV vs. RI, $r = 0.176$, $p = 0.0053$) correlated with each other, suggesting that coexistence of arterial stiffness and vascular resistance acts as a risk factor for impaired peripheral circulation in lower-leg arteries in diabetic patients even though they have a normal ABI.

3.2. *Peripheral circulation and nephropathy*

Simple linear regression analysis demonstrated that the entire group of diabetic patients ($n = 250$) did not show a significant

Table 1 – Clinical characteristics and vascular parameters in all subjects

	Nondiabetic subjects	Diabetic patients	p-Value
Number	40	250	
Male sex (%)	21 (52.5)	144 (57.6)	0.6652
Age (years)	59.8 ± 6.5	61.5 ± 6.6	0.1243
BMI (kg/m^2)	22.6 ± 1.9	23.6 ± 3.5	0.0821
Duration of diabetes (years)	–	12.3 ± 8.4	–
Treatment (D/OHD/I)	–	16/86/148	–
FPG (mmol/l)	5.35 ± 0.39	6.97 ± 1.61	<0.0001
HbA1c (%)	4.7 ± 0.4	8.2 ± 1.3	<0.0001
TC (mmol/l)	4.92 ± 0.57	4.85 ± 0.80	0.5898
HDL-C (mmol/l)	1.43 ± 0.42	1.24 ± 0.35	0.0024
TGs (mmol/l)	1.14 ± 0.31	1.40 ± 0.75	0.0261
Blood pressure (mmHg)			
Systolic	122 ± 7	128 ± 17	0.0230
Diastolic	75 ± 8	71 ± 10	0.0202
ACEI and/or ARB (%)	–	87 (34.8)	–
Smokers (%)	15 (37.5)	132 (52.8)	0.1038
Retinopathy (%)	–	107 (42.8)	–
Albuminuria (%)	–	92 (36.8)	–
Neuropathy (%)	–	110 (44.0)	–
eGFR (ml/min/1.73 m^2)	71.7 ± 7.2	72.9 ± 17.2	0.7401
ABI	1.12 ± 0.09	1.11 ± 0.09	0.4977
baPWV (cm/s)	1290 ± 113	1758 ± 359	<0.0001
Heart rate (bpm)	71 ± 9	72 ± 11	0.6298
Flow volume (ml/min)			
Total	90.6 ± 19.3	75.6 ± 26.0	0.0005
Systolic	84.5 ± 15.8	81.2 ± 19.7	0.3203
Early diastolic	−10.8 ± 7.7	−13.4 ± 9.3	0.0963
Late diastolic	17.0 ± 6.9	7.8 ± 9.1	<0.0001
Resistive index	0.969 ± 0.026	1.017 ± 0.045	<0.0001

Data are expressed as n (%) or means ± S.D. D, diet; OHD, oral hypoglycemic drugs; I, insulin.

Table 2 – Diabetic patients grouped into four subgroups with or without albuminuria and renal insufficiency according to the levels of urinary albumin excretion rate of ≥20 or <20 μg/min and estimated glomerular filtration rate of <60 or ≥60 ml/min/1.73 m²

	Normoalbuminuria		Albuminuria	
	Without renal insufficiency	With renal insufficiency	Without renal insufficiency	With renal insufficiency
eGFR (ml/min/1.73 m²)	79.3 ± 12.9	51.6 ± 6.9	78.7 ± 12.7	47.2 ± 9.8
Overt proteinuria (%)	–	–	13 (21.0)	23 (76.7)
Number	136	22	62	30
Male sex (%)	75 (55.1)	9 (40.9)	40 (64.5)	20 (66.7)
Age (years)	61.1 ± 6.7	64.0 ± 5.5	60.2 ± 6.1	64.6 ± 6.5^{g}
BMI (kg/m²)	23.1 ± 3.2	24.5 ± 4.5	24.2 ± 3.5	24.0 ± 3.7
Duration of diabetes (years)	10.4 ± 7.6	11.3 ± 6.5	13.5 ± 8.5	19.1 ± 9.2^{c,e,g}
Treatment (D/OHD/I)	7/52/77	2/5/15	5/21/36	2/8/20
FPG (mmol/l)	6.86 ± 1.55	7.16 ± 1.29	7.16 ± 1.83	6.99 ± 1.67
HbA1c (%)	8.3 ± 1.4	8.4 ± 1.0	8.2 ± 1.2	8.1 ± 1.4
TC (mmol/l)	4.80 ± 0.75	4.94 ± 0.70	4.90 ± 0.81	4.95 ± 1.02
HDL-C (mmol/l)	1.29 ± 0.34	1.31 ± 0.49	1.16 ± 0.32	1.14 ± 0.32
TGs (mmol/l)	1.26 ± 0.71	1.54 ± 1.07	1.57 ± 0.63	1.61 ± 0.73
Blood pressure (mmHg)				
Systolic	123 ± 14	124 ± 15	134 ± 19^{c}	143 ± 17^{c,f}
Diastolic	71 ± 9	69 ± 9	72 ± 10	75 ± 11
ACEI and/or ARB (%)	32 (23.5)	6 (27.3)	28 (45.2)^{a}	21 (70.0)^{b}
Smokers (%)	65 (47.8)	11 (50.0)	38 (61.3)	18 (60.0)
Retinopathy (%)	41 (30.1)	6 (27.3)	36 (58.1)^{b}	24 (80.0)^{b}
Neuropathy (%)	52 (38.2)	8 (36.4)	35 (56.5)	15 (50.0)
ABI	1.11 ± 0.08	1.09 ± 0.08	1.12 ± 0.08	1.11 ± 0.11
baPWV (cm/s)	1668 ± 330	1714 ± 407	1873 ± 367^{b}	1961 ± 289^{c}
Heart rate (bpm)	71 ± 12	71 ± 10	73 ± 10	75 ± 11
Flow volume (ml/min)				
Total	75.8 ± 25.7	84.3 ± 16.5	79.5 ± 27.1	59.8 ± 25.0^{a,e,h}
Systolic	80.0 ± 18.9	86.2 ± 14.4	87.3 ± 20.8	70.6 ± 19.6^{d,h}
Early diastolic	−13.1 ± 9.6	−10.2 ± 6.3	−15.2 ± 9.7	−13.7 ± 8.5
Late diastolic	8.9 ± 9.3	8.3 ± 6.5	7.4 ± 9.6	3.0 ± 8.0^{a}
Resistive index	1.012 ± 0.046	1.011 ± 0.037	1.020 ± 0.042	1.039 ± 0.040^{a}

Data are expressed as n (%) or means ± S.D. D, diet; OHD, oral hypoglycemic drugs; I, insulin. $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ vs. normoalbuminuric patients without renal insufficiency; $^{d}p < 0.05$, $^{e}p < 0.01$, $^{f}p < 0.001$ vs. normoalbuminuric patients with renal insufficiency; $^{g}p < 0.05$, $^{h}p < 0.01$ vs. albuminuric patients without renal insufficiency.

correlation between total flow volume and eGFR. Therefore, to clarify the role of nephropathy in impaired blood flow in lower-leg arteries, diabetic patients were classified into four subgroups with or without albuminuria and renal insufficiency. Although they showed normoalbuminuria, 22 of the 250 (8.8%) patients had renal insufficiency. Clinical characteristics and vascular parameters in those subgroups are shown in Table 2. There were no significant differences among the groups for frequency of male gender, BMI, FPG, HbA1c, TC, HDL-C, TGs, frequency of smoking habit and neuropathy. However, albuminuric patients with renal insufficiency showed the oldest age ($p = 0.0249$), longest duration of diabetes ($p < 0.0001$), and highest sBP ($p < 0.0001$), frequency of patients taking ACEI and/or ARB ($p < 0.01$), and frequency of retinopathy ($p < 0.01$) among the groups. There were no significant differences in ABI among the groups. Normoalbuminuric patients with and without renal insufficiency had similar baPWV, whereas albuminuric patients with renal insufficiency showed the highest baPWV ($p = 0.0006$) among the groups, indicating that elevated urinary albumin excretion is a possible risk factor for arterial stiffness. Waveform analysis at the popliteal artery using gated 2D-cine-PC MRI is shown in Fig. 1. Normoalbuminuric patients without renal insufficiency showed a typically triphasic waveform, which could be clearly separated into systolic, early diastolic and late diastolic phases during the cardiac cycle (Fig. 1A). Waveforms in the normoalbuminuric patients with renal insufficiency (Fig. 1B) and albuminuric patients without renal insufficiency (Fig. 1C) were similar to those in the normoalbuminuric patients without renal insufficiency, whereas albuminuric patients with renal insufficiency (Fig. 1D) showed reduced blood flow and abnormal flow reversal in late diastole, suggesting the presence of higher arterial stiffness and greater vascular resistance in lower-leg arteries. There were no significant differences in heart rate and early diastolic flow reversal among the groups. Although normoalbuminuric patients with renal insufficiency and albuminuric patients without renal insufficiency had similar flow parameters, albuminuric patients with renal insufficiency demonstrated the lowest total ($p = 0.0078$), systolic ($p = 0.0019$) and late diastolic ($p = 0.0147$) flow volumes and the greatest RI ($p = 0.0274$) among the groups, suggesting that parallel development of albuminuria and renal dysfunction is a possible risk factor for vascular resistance and impaired blood

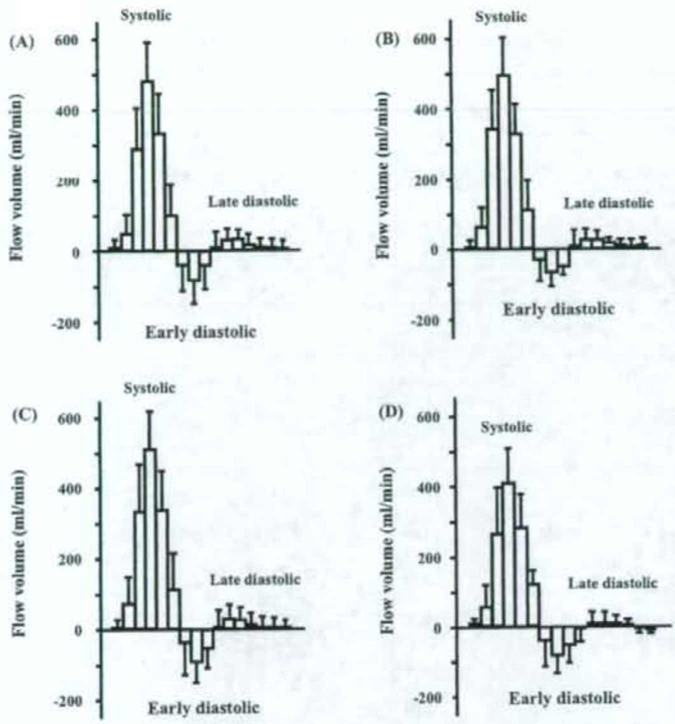

Fig. 1 – Waveform analysis at the popliteal artery in type 2 diabetic patients with normal ankle–brachial indices (ABI > 0.9) grouped into four subgroups with or without albuminuria and renal insufficiency according to the levels of urinary albumin excretion rate (AER) of ≥20 or <20 μg/min and estimated glomerular filtration rate (eGFR) of <60 or ≥60 ml/min/1.73 m^2. Data are expressed as means ± S.D. (A) Normoalbuminuric patients without renal insufficiency; (B) normoalbuminuric patients with renal insufficiency; (C) albuminuric patients without renal insufficiency; (D) albuminuric patients with renal insufficiency.

flow. To clarify the role of elevated urinary albumin excretion in peripheral circulation in lower-leg arteries, patients were classified into two subgroups with normoalbuminuria ($n = 158$) or albuminuria ($n = 92$). Simple linear regression analyses, as shown in Fig. 2, revealed that among the patients with albuminuria eGFR was correlated positively with total flow volume ($r = 0.319$, $p = 0.0020$) (Fig. 2A) and negatively with baPWV ($r = -0.232$, $p = 0.0258$) (Fig. 2B) and RI ($r = -0.308$, $p = 0.0029$) (Fig. 2C). However, there were no significant correlations between eGFR and those vascular parameters in patients with normoalbuminuria (Fig. 2D–F).

4. Discussion

4.1. *Peripheral circulation*

In the present study, waveforms at the popliteal artery in the normoalbuminuric patients with renal insufficiency and albuminuric patients without renal insufficiency were similar to those in the normoalbuminuric patients without renal insufficiency, whereas the albuminuric patients with renal insufficiency showed reduced blood flow and abnormal flow reversal in late diastole. These results suggest that albuminuric patients with renal insufficiency have higher arterial stiffness and greater vascular resistance in lower-leg arteries. There are important differences among elastic and muscular arteries and arterioles. All are impaired in diabetic patients [16,25]. Large arteries, including the aorta and its major branches, have elastic properties of the vessel wall and act as carrying vessels and blood supply reservoirs [26]. When there is a decrease in arterial elasticity, less blood can be stored in these arteries, resulting in a decrease in diastolic forward flow. The medium- and small-caliber arteries and arterioles, which have functional smooth muscles in the vessel wall, act as resistance vessels regulating blood flow to the capillaries [26]. Endothelial dysfunction and reduced lumen diameter in small vessels can increase peripheral vascular resistance [16], resulting in an abnormal flow reversal in late diastole. These vascular abnormalities can coexist in the same individual [27]. Our data demonstrated that blood flow, arterial stiffness, and vascular resistance were negatively or positively correlated with each other, suggesting that coexistence of arterial stiffness and vascular resistance acts as a risk factor for

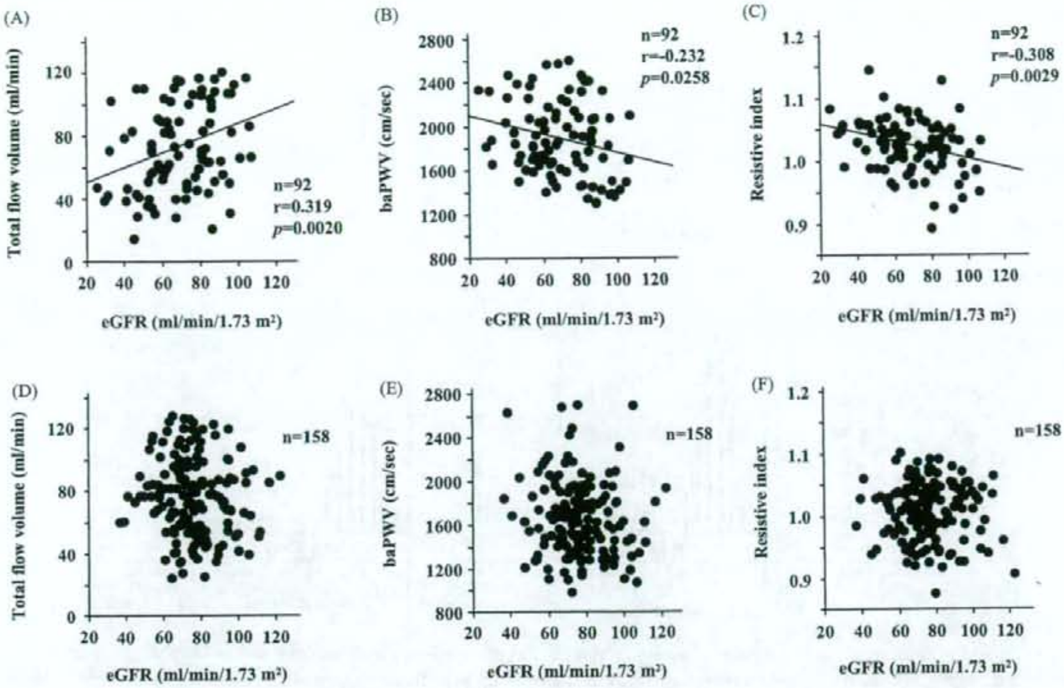

Fig. 2 – Simple linear regression analyses between estimated glomerular filtration rate (eGFR) and total flow volume, brachial–ankle pulse wave velocity (baPWV), or resistive index in lower-leg arteries in albuminuric ($n = 92$) (A–C) or normoalbuminuric ($n = 158$) (D–F) type 2 diabetic patients with normal ankle–brachial indices (ABI > 0.9).

impaired peripheral circulation in lower-leg arteries in diabetic patients even though they have a normal ABI.

4.2. *Nephropathy*

As we reported previously, when the diagnostic criterion for critical lower-limb ischemia in diabetic patients of transcutaneous oxygen tension of <50 mmHg at the dorsum of the foot was used [28], 16 of 60 (26.7%) diabetic patients had ischemic lower limbs although they had no apparent PAOD [23]. Diabetic nephropathy is a major cause of lower-limb amputation associated with peripheral vascular disorders [1]. It is desirable to ameliorate insufficient arterial blood flow before the onset of lower-limb ischemia. Endothelium actively regulates vascular tone and permeability and balance between coagulation and fibrinolysis [29]. Therefore, elevated urinary albumin excretion is regarded as a renal expression of systemic endothelial damage being extended to the whole arterial system [29]. In our present study, when the diabetic patients were classified into four subgroups with or without albuminuria and renal insufficiency, albuminuric patients with renal insufficiency showed the highest arterial stiffness, greatest vascular resistance, and lowest blood flow in lower-leg arteries among the groups. These results suggest that parallel development of albuminuria and renal dysfunction is a possible risk factor for arterial stiffness, vascular resistance, and impaired blood flow in lower-leg arteries. Our data revealed that renal function was correlated positively with blood flow and negatively with arterial stiffness and vascular resistance in lower-leg arteries among the patients with albuminuria, but not in those with normoalbuminuria. Alterations in capillaries, glomeruli and tubulointerstitial compartments of the kidney can increase renal vascular resistance assessed by duplex Doppler sonography [7], and those abnormalities associate with the severity of systemic atherosclerosis [8,9]. Therefore, although we did not measure intrarenal vascular resistance in the present study, we surmise that increased vascular resistance at the popliteal artery may associate with the severity of diabetic nephropathy.

Nephropathy in patients with type 2 diabetes is more heterogeneous than that in type 1 diabetes. Type 2 diabetic patients can develop renal impairment in the absence of increased albuminuria, and those conditions are initially developed during the prediabetic state secondary to age, hypertension and other factors [30]. In the United States, 30% of newly diagnosed type 2 diabetic patients showed renal insufficiency although they did not have retinopathy and albuminuria [31]. Our study revealed that 8.8% of our patients had normoalbuminuric renal insufficiency. It has been reported that risk for progression of renal failure or death in diabetic patients with normoalbuminuric renal insufficiency is lower than in those with albuminuric renal insufficiency [32]. In the present study, arterial stiffness, vascular resistance