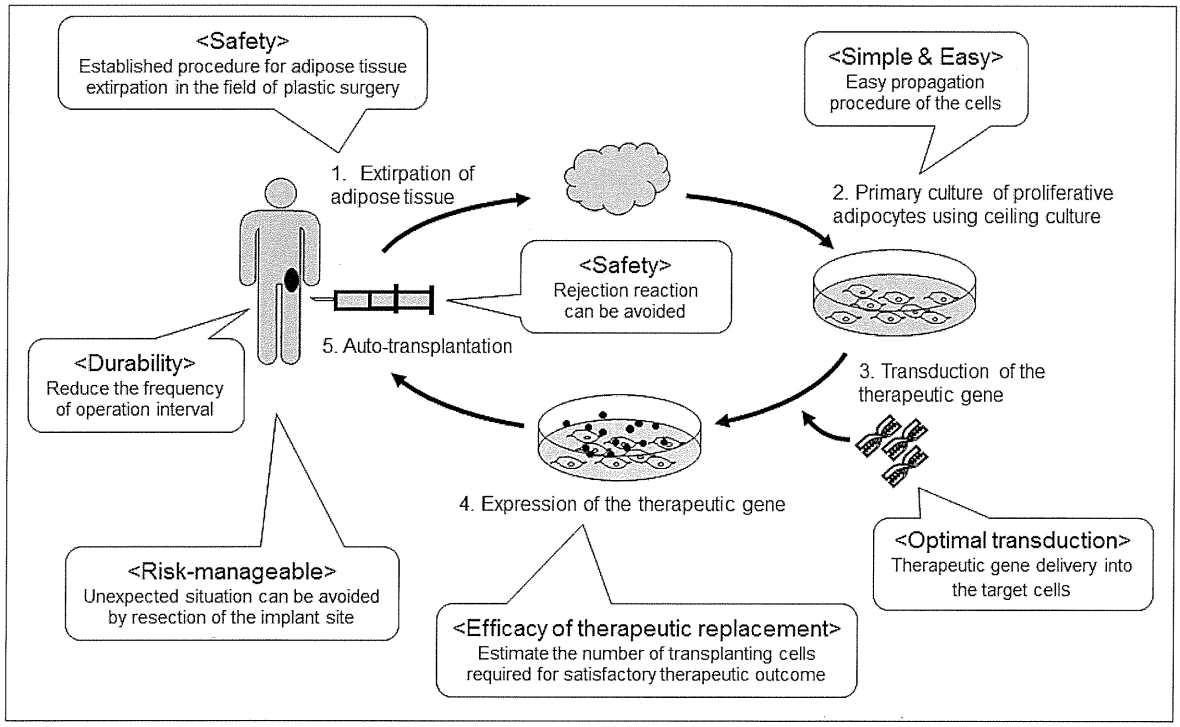


図4 Enzyme replacement therapy by *ex vivo* manipulated ccdPA
Characteristics of therapeutic strategy using adipose tissue-derived cells are depicted.

ら[11]，再生医療用の細胞として期待され，中枢神経の再生，脂肪萎縮症，クローン病などのへの研究が行われている[12~14]．現在遺伝子治療の適応となる先天性疾患は治療蛋白の局在から二種類に大別される．すなわち，機能傷害部位が細胞内で，それを補完しようとするものと，インスリンのように血中(細胞外)に分泌させるものである．前者に分類される先天性免疫不全症や網膜疾患の場合には造血幹細胞や網膜細胞に機能回復のための治療遺伝子を導入する必要がある[15,16]．一方後者の場合，標的細胞には基本的に制限はない．さらに *ex vivo* 治療法は理論的に分泌蛋白質の産生量をコントロールすることが可能であるという点で *in vivo* 法よりもすぐれ，この観点から *in vitro* で充分な増殖能を保持する細胞，寿命が長い細胞，がん化リスクの低い，といった安全性の高い細胞が候補となる．これまで *ex vivo* 遺伝子治療研究で利用または検討されている細胞は，造血幹細胞，T 細胞，線維芽細胞，肝細胞，ケラチノサイトなどである[17,18]．しかし，これらの細胞は採取できる細胞数，増殖能，組織採取時の侵襲性の問題から制約が多く，期待されるほどの成果は得られておらず，細胞医薬品としての応用研究は進んでいない．

筆者らは，脂肪細胞が有する移植材料としてのすぐれた特長に加え，脂肪細胞が生命維持に重要な役割を果たすサイトカインの分泌細胞であること，細胞の寿命が長いこと，さらに脂肪細胞のがん化リスクが低いことから，血清酵素の欠損による数多くの難治性疾患に対して，*ex vivo* での遺伝子治療により欠損酵素を分泌装置としての脂肪細胞調製技術確立と治療目的遺伝子を組み込んだヒト脂肪細胞の細胞医薬品開発を行っている．

最初の治療対象疾患として，先天性脂質代謝異常症の一つである家族性レシチンコレステロール：アシルトランスフェラーゼ(LCAT)欠損症を選択した．その理由として，臨床的に LCAT の過剰症が報告されていないこと，一方，その予後を規定すると考えられる腎不全は，健常人の LCAT 濃度の 10%程度未満であると合併すると推察され，目標とする補充量があらかじめ設定できていることがあげられる．これまで，ccdPA を用いて検討を重ねた結果，7 日間の初代培養で得られる ccdPA が，14 日間の初代培養で得られる細胞にくらべて，レトロウイルスベクターによる LCAT 遺伝子の導入特性がすぐれていることを明らかにし

た[6,19]．ヒト脂肪組織からのccdPAの調製からLCAT遺伝子導入，拡大培養，移植細胞製剤調製までのGMP製造法を確立し(**図4**)，非臨床試験成績とともに千葉大学から遺伝子治療臨床研究実施計画が厚生労働省に申請され[20]，2013年5月に遺伝子治療臨床研究実施に関する妥当性について承認を得ている．

このように，ccdPAは酵素補充療法を目的とした*ex vivo*遺伝子治療用の分泌細胞装置として有望であると考えられ，脂肪組織由来の細胞が，ASCのみならず，新たな難治性疾患治療用の細胞として医療に大きく貢献することが期待される．現在は，ほかの疾患への応用研究も進めている．

## おわりに

ASCが多分化能を示すことは多くの論文で報告されており，わが国でも多くの幹細胞臨床研究が行われている．筆者らの検討においては，ccdPAはASCと同程度の骨，軟骨への分化能を示し，脂肪分化能については，ASCよりもすぐれていた．これらの細胞の分化特性の違いは，ccdPA，ASCの個々の細胞における分化に関連した遺伝子発現やエピジェネティックな要因または外的要因，あるいは両方に起因することが考えられる．Rodeheffer ら[21]もSVFの脂肪細胞への分化率が低いことを報告し，SVFには分化を阻害する細胞または因子が存在するのではないかと推測している．このように，脂肪分化特性が異なる細胞が同じ脂肪組織から得られることは，脂肪組織の構築や脂肪分化シグナルの研究にも重要な生体試料となり得ることを示唆している．さらにこれらの特性を明らかにすることで，ccdPAとASCの細胞治療における目的に応じた使い分けができるようになる可能性がある．

これまで脂肪組織は形成外科領域において組織欠損部位などの修復に多用されてきた．この臨床実績は脂肪組織が移植用生体材料として安全性が高いことを示している．事実，脂肪細胞はがんの報告がまれであり，さらに近年，脂肪分化能ががん化に抑制的に働くことが基礎研究成果から示唆されている[22]．今後は脂肪組織由来の細胞についてその特性に関する基礎研究が進み，分子・細胞レベルで脂肪組織移植，さらには個々の細胞の再生細胞治療における移植効率や安全性に関わるメカニズムが明らかになることを期待している．これらの臨床基礎研究成果がさらに生体材料としての脂肪組織の理解を深めることになると考えられる．

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別刷請求先 : 麻生雅是
〒 260-0856 千葉市中央区亥鼻 1-8-5 千葉大亥鼻イノベーション
セルジェンテック株式会社
E-mail : masoph@cellgentech.com

Original Article

# Arterial Wall Elasticity Measured Using the Phased Tracking Method and Atherosclerotic Risk Factors in Patients with Type 2 Diabetes

Michiaki Miyamoto[1], Kazuhiko Kotani[1], Kenta Okada[2], Akihiko Ando[2], Hideyuki Hasegawa[3,4], Hiroshi Kanai[4,3], Shun Ishibashi[2], Toshiyuki Yamada[1], Nobuyuki Taniguchi[1]

[1]Department of Clinical Laboratory Medicine, Jichi Medical University, Tochigi, Japan
[2]Division of Endocrinology and Metabolism, Department of Medicine, Jichi Medical University, Tochigi, Japan
[3]Graduate School of Biomedical Engineering, Tohoku University, Sendai, Japan
[4]Graduate School of Engineering, Tohoku University, Sendai, Japan

*Aim*: The aim of this study was to investigate the relationship between atherosclerotic manifestations and brachial and radial arterial wall elasticity (AWE) measured using the phased tracking method in patients with type 2 diabetes mellitus (T2DM).
*Methods*: This study included T2DM patients ($n$=220, mean age 59 years) without a history of stroke or coronary artery disease. The brachial AWE, radial AWE, carotid mean intima-media thickness (IMT), max-IMT and flow-mediated vasodilation (FMD) were measured. The patients were classified according to the number of atherosclerotic risk factors, including obesity, dyslipidemia and hypertension. Group 1 included T2DM patients only, group 2 included patients with two risk factors, group 3 included patients with three risk factors and group 4 included patients with four risk factors. The patients were also divided into two groups according to microangiopathic complications, including retinopathy and nephropathy. The between-group differences were analyzed.
*Results*: The brachial AWE (548, 697, 755 and 771 kPa for groups 1, 2, 3 and 4, respectively) and radial AWE (532, 637, 717 and 782 kPa for groups 1, 2, 3 and 4, respectively) significantly increased in association with an increasing number of risk factors. The brachial AWE and radial AWE were significantly higher in the patients with microangiopathic complications than in those without microangiopathic complications (brachial AWE 797 and 694 kPa and radial AWE 780 and 660 kPa, respectively). Receiver operating characteristic curve analyses revealed that, for brachial AWE and radial AWE, the area under the curve was equal to the max-IMT and higher than the mean-IMT and FMD.
*Conclusions*: Upper limb AWE measurement can reflect the degree of atherosclerosis risk overload and may be useful for evaluating vascular complications in T2DM patients.

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**Key words:** Ultrasonography, Carotid intima-media thickness, Flow-mediated vasodilation

## Introduction

Type 2 diabetes mellitus (T2DM) is associated with atherosclerosis, which leads to various vascular complications, including macroangiopathies, such as coronary artery disease and strokes, and microangiopathies, such as retinopathy and nephropathy[1-5]. The addition of obesity, dyslipidemia and hypertension to T2DM accelerates the progression of atherosclerosis and increases the risk of cardiovascular disease[3, 6-8]. Managing these atherosclerotic risk factors can prevent atherosclerotic progression[9]; therefore, it is important to evaluate a patient's atherosclerotic risk overload prior to the occurrence of cardiovascular events[10, 11]. Several atherosclerotic markers, such as the carotid intima-media thickness (IMT) and flow-mediated

Address for correspondence: Kazuhiko Kotani, Department of Clinical Laboratory Medicine, Jichi Medical University, 3311-1 Yakushiji, Shimotsuke-City, Tochigi 329-0498, Japan
E-mail: kazukotani@jichi.ac.jp
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vasodilation (FMD), are useful for assessing atherosclerotic risks, although they do not always predict cardiovascular risks among T2DM patients[12, 13].

Recently, a noninvasive, transcutaneous, ultrasonic technique using the phased tracking method was developed to evaluate arterial wall elasticity (AWE). This method is believed to be able to detect atherosclerotic changes in association with regional tissue composition[14-16]. During a single heartbeat, the displacement of each point of the arterial wall from the luminal surface to the adventitia is tracked along an ultrasonic beam, and minute changes in the thickness of different layers can be detected, allowing the elastic modulus to provide information on regional tissue composition[14-16]. The AWE is expressed as the mean elastic modulus of the entire arterial wall of a regional artery. Because it is an easy-to-use and noninvasive method, measuring the AWE using the phased tracking method is suitable as an atherosclerotic screening test.

The phased tracking method has been used to demonstrate that soft tissue, such as soft plaques with a lipid core within the arteries have a low AWE, while a high AWE is seen in stiff tissue, such as lesions where calcification and proliferation of vascular smooth muscle and collagen exist[14]. The carotid AWE has been reported to be significantly associated with atherosclerotic markers, such as the carotid max-IMT and pulse wave velocity, in T2DM patients[15], whereas the radial AWE is significantly associated with the serum C-reactive protein level, a chronic inflammatory index, in healthy subjects[16, 17]. Therefore, measuring the AWE may be useful for evaluating the degree of atherosclerotic overload. However, currently, only a few studies have evaluated the AWE measured according to the phased tracking method in different arteries or different populations[15, 16]; therefore, further clinical data are required to establish the clinical utility of AWE measurement using this method. For this purpose, we investigated the relationship between the degree of atherosclerotic risk overload and the AWE in addition to the differences in AWE between patients with and without microangiopathic complications in order to determine whether upper limb AWE measurement can be used as new approach for evaluating atherosclerosis.

## Aim

The brachial AWE, radial AWE and other ultrasonic atherosclerosis-related markers, including the carotid IMT and FMD, and conventional atherosclerotic risk factors were measured in T2DM patients without a history of stroke or coronary artery disease. This study investigated whether brachial and radial AWE values are correlated with the number of atherosclerotic risk factors in T2DM patients. Furthermore, we investigated the brachial and radial AWE in patients with microangiopathic complications because patients with diabetic microangiopathic complications, particularly those in the advanced stage, constitute a special population with a high risk for cardiovascular events[18-20].

## Methods

### Subjects

A total of 220 patients with T2DM (men, 57%; mean ± standard deviation [SD] age, 59 ± 11 years) were sequentially included in this cross-sectional hospital-based study. The Ethics Committee at Jichi Medical University approved the study, and each patient gave their informed consent. Patients with a history of stroke or coronary artery disease were excluded. All of the study patients were stable and receiving antidiabetic treatments, such as diet therapy, oral medications and insulin. T2DM was diagnosed based on the World Health Organization (WHO) and American Diabetes Association (ADA) criteria: a fasting plasma glucose level of ≥ 126 mg/dL and/or a 2-hour plasma glucose level after a 75-g oral glucose tolerance test of ≥ 200 mg/dL[21, 22].

The diagnosis of retinopathy was determined by a trained ophthalmologist using indirect ophthalmoscopic examinations based on the presence of clinical features in the fundus in both eyes[23]. Advanced-stage retinopathy was defined as proliferative retinopathy, severe nonproliferative retinopathy, post vitreous surgery retinopathy or post panretinal photocoagulation[23]. The renal function was assessed using the estimated glomerular filtration rate (eGFR), which was calculated according to the glomerular filtration rate equation for Japanese subjects[24]. Patients with an eGFR of ≤ 30 mL/min/1.73 $m^2$ and/or macroalbuminuria (a urinary albumin-to-creatinine ratio [ACR] of ≥ 300 mg/g creatinine [Cr]) were classified as having advanced stage disease[25-27]. Nephropathy was defined as an eGFR of ≤ 60 mL/min/1.73 $m^2$ and/or microalbuminuria (ACR ≥ 30 mg/g Cr)[26, 27].

The patients were divided into four groups based on the number of atherosclerotic risk factors, including obesity, dyslipidemia and hypertension[3, 6]. Group 1 included patients with T2DM only, group 2 included patients with two risk factors, group 3 included patients with three risk factors and group 4 included patients with all four risk factors. The patients were further classified into two groups: those with and

without microangiopathic complications, including advanced-stage retinopathy or nephropathy.

**Physical Examinations**

Smoking habits were determined in interviews conducted by doctors, and smokers were defined as current smokers. The body mass index (BMI) was calculated as the weight divided by the square of the body height while wearing light clothes. Obesity was defined as a BMI of ≥ 25.0 kg/m$^2$ based on the Japan Society for the Study of Obesity criteria[28]. Blood pressure was measured twice in the supine position with a minimum of five minutes between measurements, and the mean value was recorded. Hypertension was defined as a systolic blood pressure of ≥ 140 mmHg and/or a diastolic blood pressure of ≥ 90 mmHg and/or the current use of antihypertensive agents[29].

**Laboratory Measurements**

Blood samples were drawn in the morning after a 12-hour fast. The level of hemoglobin A1c (HbA1c) was measured using high-performance liquid chromatography. The present study used the value of HbA1c (%) estimated as a National Glycohemoglobin Standardization Program (NGSP) equivalent value (%) (HbA1c [Japan Diabetes Society: JDS] + 0.4%)[30]. The levels of blood glucose, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides and Cr were determined enzymatically. Dyslipidemia was defined as an LDL-C level of ≥ 140 mg/dL, an HDL-C level of < 40 mg/dL, a triglyceride level of ≥ 150 mg/dL and/or the current use of statins or fibrates[31]. The ACR in spot urine was also assessed, and values of albuminuria surpassing the sensitivity of the ACR measurement were recorded as 300 mg/g Cr.

Using B-mode ultrasound imaging with a 5- to 10-MHz linear transducer (ALOKA, SSD-$\alpha$10, Tokyo, Japan), the mean-IMT and max-IMT were evaluated bilaterally at the common carotid artery, bifurcation and internal carotid arteries[32, 33]. The mean-IMT was measured in the bilateral common carotid arteries (excluding the bulbus), and the levels at two or more measurement points were averaged[32, 33]. The max-IMT was measured at the greatest IMT in the bilateral common carotid arteries, the bulbus and the internal carotid arteries[32].

For the FMD measurements, the patients fasted and did not use tobacco for at least eight hours before measurement[34, 35]. The FMD was assessed in the patient's right brachial artery above the antecubital fossa in the supine position in a quiet, temperature-controlled room. The diameter of the brachial artery was measured continuously using B-mode ultrasound. A cuff was placed on the forearm and, after measuring the baseline diameter, arterial occlusion was induced by cuff inflation to a pressure of 200 mmHg or 50 mmHg above the systolic blood pressure for five minutes. When the cuff was released, the FMD was calculated as the maximum percent increase in the diameter during hyperemia compared with the baseline diameter.

**Measurement of Arterial Wall Elasticity Using the Phased Tracking Method**

The brachial AWE and radial AWE were evaluated using the phased tracking method[14-16]. The brachial and radial arteries were assessed above the antecubital fossa and approximately 5 cm proximal to the wrist with the palm turned upward, respectively, in the supine position in a quiet, temperature-controlled room. The arteries were scanned in the longitudinal plane using B-mode ultrasound imaging with an 8- to 16-MHz linear array transducer (DIASUS, Dynamic Imaging Ltd., Livingston, UK). Ultrasound was used to record changes in the thickness of the artery during a single heartbeat. The arterial wall was divided into layered blocks with a depth of 312 $\mu$m and a width of 200 $\mu$m for the entire wall, and the elastic modulus of each layer ($E_\theta$ [Pascal: Pa]) was calculated using the equation $E_\theta = (1/2) \times (r_0/h_0 + 1) \times (\Delta P_{max}/\varepsilon_r)$, where $\varepsilon_r = \Delta h_{max}/h_0$, $\Delta h_{max}$ is the maximum decrease in the thickness of the 312-$\mu$m layer during one heartbeat, $\Delta P_{max}$ is the pulse pressure and $h_0$ and $r_0$ are the initial thicknesses of the layer and radius of the vessel at end-diastole, respectively. The elastic modulus was determined for each 312-$\mu$m layer, and the AWE was expressed as the mean level of all layers. The AWE was determined in the bilateral brachial and radial arteries, and the bilateral averages of each artery were used for all analyses. All examinations were performed by the same trained physician. The intraobserver coefficients of variation for the brachial AWE and radial AWE values were 9.3% and 10.9%, respectively.

**Statistical Analyses**

The data are presented as the mean ± SD, median (interquartile range) or number (%). The between-group differences of each parameter were compared using a one-way analysis of variance (ANOVA) with a multiple comparison test or the chi-square test. ANOVA adjusted for confounding factors, such as age, sex, the smoking status, the Cr level and ACR, was performed to determine the between-group differences in atherosclerotic parameters, such as the brachial AWE, radial AWE, mean-IMT, max-IMT and FMD. The between-group differences in atherosclero-

**Table 1.** Patient profiles for each group based on the number of atherosclerotic risk factors

| Parameter | All ($n$=220) | Group 1 ($n$=22) | Group 2 ($n$=65) | Group 3 ($n$=76) | Group 4 ($n$=57) | $p$-value |
|---|---|---|---|---|---|---|
| Male (%) | 126 (57%) | 13 (59%) | 40 (62%) | 40 (53%) | 33 (58%) | 0.76 |
| Age (years) | 59±11 | 57±14 | 61±9 | 60±11 | 58±10 | 0.32 |
| Body mass index (kg/m$^2$) | 25±6 | 20±2 | 23±3$^*$ | 26±6$^{*,\S}$ | 30±4$^{*,\S,\dagger}$ | <0.01 |
| Smoking (%) | 47 (21%) | 6 (27%) | 14 (22%) | 18 (24%) | 9 (16%) | 0.62 |
| SBP (mmHg) | 128±15 | 117±13 | 125±15 | 129±15$^*$ | 134±14$^{*,\S}$ | <0.01 |
| DBP (mmHg) | 77±9 | 72±8 | 76±9 | 77±9 | 80±9$^{*,\S}$ | <0.01 |
| Anti-HT agents (%) | 112 (51%) | 0 (0%) | 15 (23%) | 45 (59%)$^{*,\S}$ | 52 (91%)$^{*,\S,\dagger}$ | <0.01 |
| RAS inhibitors (%) | 104 (47%) | 0 (0%) | 13 (20%) | 42 (55%)$^{*,\S}$ | 49 (86%)$^{*,\S,\dagger}$ | <0.01 |
| CCB (%) | 69 (31%) | 0 (0%) | 8 (12%) | 27 (36%)$^{*,\S}$ | 34 (60%)$^{*,\S,\dagger}$ | <0.01 |
| $\beta$ blockers (%) | 11 (5%) | 0 (0%) | 1 (2%) | 7 (9%) | 3 (5%) | 0.13 |
| Diuretics (%) | 28 (13%) | 0 (0%) | 4 (6%) | 11 (14%)$^{*,\S}$ | 13 (23%)$^{*,\S,\dagger}$ | 0.01 |
| Glucose (mg/dL) | 135±42 | 127±54 | 134±43 | 136±42 | 139±38 | 0.67 |
| Hemoglobin A1c (%) | 7.9±2.0 | 7.9±2.3 | 7.9±2.3 | 8.0±2.0 | 7.7±1.6 | 0.87 |
| Antidiabetic agents (%) | 158 (72%) | 13 (59%) | 41 (63%) | 57 (75%) | 47 (82%) | 0.05 |
| OHA (%) | 109 (50%) | 7 (32%) | 29 (45%) | 43 (57%) | 30 (53%) | 0.16 |
| Insulin treatment (%) | 49 (22%) | 6 (27%) | 12 (18%) | 14 (18%) | 17 (30%) | 0.34 |
| Retinopathy (%) | 80 (36%) | 2 (9%) | 20 (31%) | 30 (39%)$^*$ | 28 (49%)$^{*,\S}$ | 0.01 |
| Non-advanced stage (%) | 42 (19%) | 1 (5%) | 12 (18%) | 13 (17%) | 16 (21%) | 0.10 |
| Advanced stage (%) | 38 (17%) | 1 (5%) | 8 (12%) | 17 (22%) | 12 (21%) | 0.14 |
| LDL-cholesterol (mg/dL) | 109±32 | 106±22 | 109±31 | 114±33 | 104±33 | 0.37 |
| HDL-cholesterol (mg/dL) | 57±19 | 78±25 | 57±18$^*$ | 56±16$^*$ | 52±18$^*$ | <0.01 |
| Triglycerides (mg/dL) | 108 (81-160) | 58 (50-84) | 104 (65-126)$^*$ | 110 (83-171)$^*$ | 153 (100-194)$^{*,\S}$ | <0.01 |
| Anti-HL agents (%) | 87 (40%) | 0 (0%) | 17 (26%) | 34 (45%)$^{*,\S}$ | 36 (63%)$^{*,\S,\dagger}$ | <0.01 |
| Statins (%) | 79 (36%) | 0 (0%) | 15 (23%) | 33 (43%)$^{*,\S}$ | 31 (54%)$^{*,\S}$ | <0.01 |
| Fibrates (%) | 8 (4%) | 0 (0%) | 2 (3%) | 1 (1%) | 5 (9%) | 0.10 |
| Cr (mg/dL) | 0.68 (0.59-0.80) | 0.66 (0.53-0.80) | 0.70 (0.59-0.79) | 0.69 (0.59-0.84) | 0.66 (0.57-0.79) | 0.71 |
| eGFR (mL/min/1.73 m$^2$) | 83±24 | 88±25 | 84±23 | 80±26 | 84±21 | 0.45 |
| ACR (mg/g Cr) | 16 (8-86) | 10 (7-23) | 17 (10-87) | 17 (9-84) | 21 (9-143) | 0.08 |
| Nephropathy (%) | 100 (45%) | 5 (23%) | 27 (42%) | 39 (51%) | 29 (51%) | 0.08 |
| Non-advanced stage (%) | 70 (32%) | 4 (18%) | 17 (26%) | 29 (38%) | 20 (35%) | 0.21 |
| Advanced stage (%) | 30 (14%) | 2 (9%) | 10 (15%) | 10 (13%) | 9 (16%) | 0.58 |
| Mean-IMT (mm) | 0.72±0.12 | 0.70±0.13 | 0.70±0.09 | 0.74±0.14 | 0.74±0.11 | 0.15 |
| Max-IMT (mm) | 1.60±0.84 | 1.31±0.74 | 1.53±0.70 | 1.68±0.92 | 1.70±0.89 | 0.21 |
| FMD (%) | 2.4±1.9 | 3.4±2.9 | 2.4±1.5 | 2.4±1.9 | 2.1±1.6 | 0.05 |
| Brachial AWE (kPa) | 721±217 | 548±141 | 697±200$^*$ | 755±222$^*$ | 771±221$^*$ | <0.01 |
| Radial AWE (kPa) | 692±222 | 532±149 | 637±207 | 717±206$^*$ | 782±238$^{*,\S}$ | <0.01 |

SBP: systolic blood pressure; DBP: diastolic blood pressure; Anti-HT: antihypertensive; RAS: renin-angiotensin system; CCB: calcium channel blockers; OHA: oral antihyperglycemic agents; LDL: low-density lipoprotein; HDL: high-density lipoprotein; Anti-HL: antihyperlipidemic; Cr: creatinine; eGFR: estimated glomerular filtration rate; ACR: urinary albumin-to-Cr ratio; IMT: intima-media thickness; FMD: flow-mediated vasodilation; AWE: arterial wall elasticity; Pa: Pascal. The patients were divided into four groups based on the number of the following atherosclerotic risk factors: diabetes mellitus, obesity, dyslipidemia and hypertension. Group 1 included patients with diabetes mellitus only, group 2 included patients with two risk factors, group 3 included patients with three risk factors and group 4 included patients with all four risk factors. The data are presented as the mean±standard deviation, median (interquartile range) or number (%). The levels of triglycerides and Cr and the ACR values were log-transformed due to their skewed distributions. The $p$-values were determined using a one-way analysis of variance with multiple comparison tests or the chi-square test with residual tests. Significance level: $^*p<0.05$ vs. group 1; $^{\S}p<0.05$ vs. group 2; $^{\dagger}p<0.05$ vs. group 3.

sis parameters adjusted for antidiabetic medications, antihypertensive medications, antihyperlipidemic medications, retinopathy and nephropathy, in addition to age, sex, the smoking status and the ACR, were also analyzed. Additionally, the groups with and without microangiopathic complications were compared using

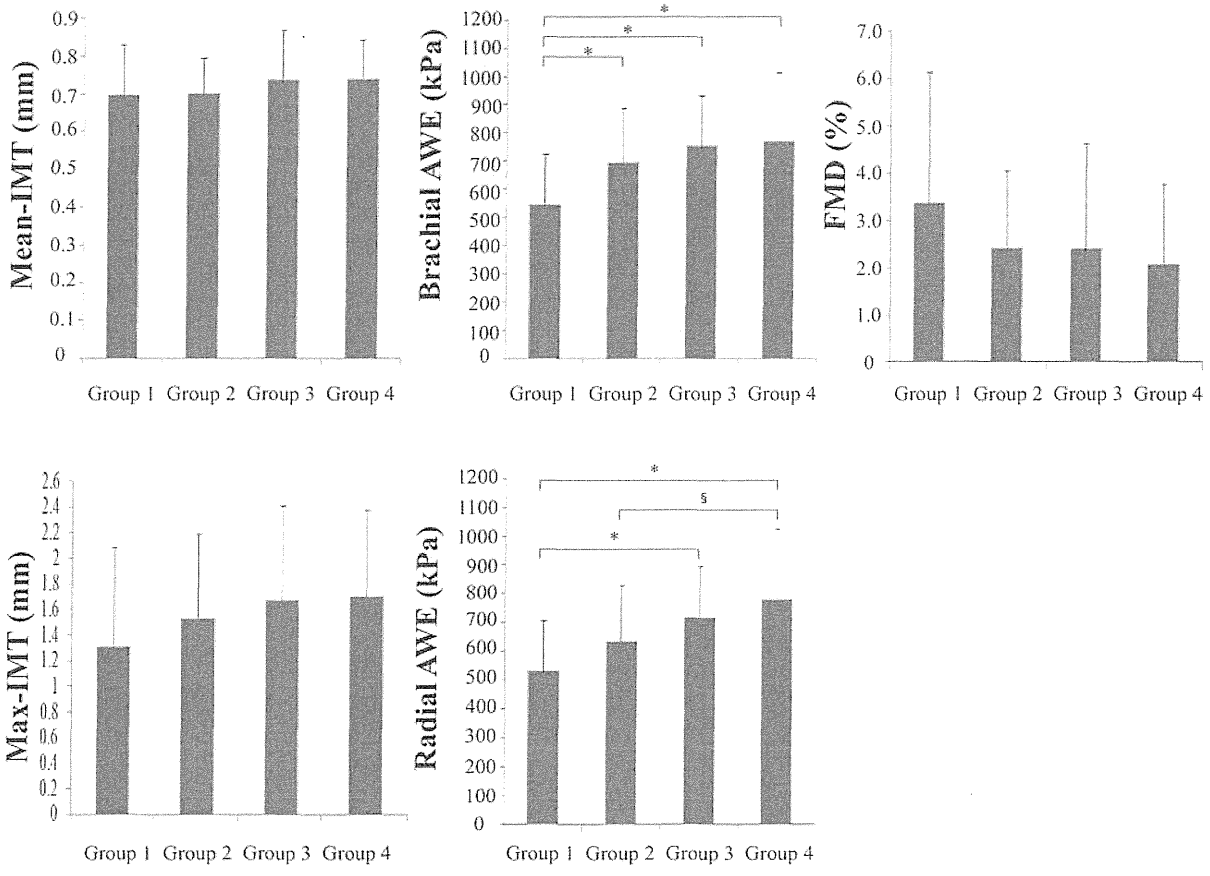


**Fig. 1.** Differences between the groups classified by the number of atherosclerotic risk factors. Group 1 included patients with diabetes mellitus only, group 2 included patient with two risk factors, group 3 included patients with three risk factors and group 4 included patients with all four risk factors. IMT: intima-media thickness; AWE: arterial wall elasticity; Pa: Pascal; FMD: flow-mediated vasodilation. Significance level: $^{*}p<0.05$ vs. group 1; $^{\S}p<0.05$ vs. group 2; $^{\dagger}p<0.05$ vs. group 3.

Student's *t*-test and the chi-square test. Receiver-operating characteristic (ROC) curve analyses of the mean-IMT, max-IMT, brachial AWE, radial AWE and FMD in the patients with microangiopathic complications were performed, and the areas under the curve (AUCs) were compared[36].

In all analyses, the log-transformed values of the levels of triglycerides and Cr and the ACR were used due to their skewed distributions. A value of $p<0.05$ was considered to be significant. All statistical analyses, except for the comparisons of the AUC, were performed using the Dr. SPSS II version 11 software program (SPSS Inc., Tokyo, Japan). The AUC comparisons were made using the StatFlex version 6 software package (Artech, Co., Ltd., Osaka, Japan).

## Results

The patient characteristics of each group based on the number of atherosclerotic risk factors are shown in **Table 1** and **Fig. 1**. As the number of atherosclerotic risk factors increased, the number of patients with retinopathy and nephropathy tended to increase. In addition, the mean-IMT and max-IMT values showed a tendency to increase from groups 1 to 4. The FMD tended to decrease from groups 1 to 4.

The brachial AWE and radial AWE values significantly increased from groups 1 to 4. The brachial AWE value was significantly higher in groups 2, 3 and 4 than in group 1. The radial AWE value was significantly higher in group 4 than in groups 1 and 2, and the radial AWE value was significantly higher in group 3 than in group 1.

After adjusting for age, sex, the smoking status, the Cr level and the ACR, significant between-group differences were found in brachial AWE ($p<0.01$) and radial AWE ($p<0.01$), and a marginally significant between-group difference was found in FMD ($p=$

**Table 2.** Patient profiles for the groups with and without microangiopathic complications

| Parameter | All ($n$=220) | Without complications ($n$=162) | With complications ($n$=58) | $p$-value |
|---|---|---|---|---|
| Male (%) | 126 (57%) | 92 (57%) | 34 (59%) | 0.81 |
| Age (years) | 59±11 | 60±11 | 58±10 | 0.17 |
| Body mass index (kg/m$^2$) | 25±6 | 25±6 | 26±5 | 0.70 |
| Smoking (%) | 47 (21%) | 34 (21%) | 13 (22%) | 0.82 |
| SBP (mmHg) | 128±15 | 127±15 | 131±14 | 0.04 |
| DBP (mmHg) | 77±9 | 76±10 | 79±9 | 0.04 |
| Anti-HT agents (%) | 112 (51%) | 73 (45%) | 39 (67%) | <0.01 |
| RAS inhibitors (%) | 104 (47%) | 67 (41%) | 37 (64%) | <0.01 |
| CCB (%) | 69 (31%) | 47 (29%) | 22 (38%) | 0.21 |
| $\beta$ blockers (%) | 11 (5%) | 8 (5%) | 3 (5%) | 0.94 |
| Diuretics (%) | 28 (13%) | 17 (10%) | 11 (19%) | 0.10 |
| Glucose (mg/dL) | 135±42 | 133±41 | 142±45 | 0.19 |
| Hemoglobin A1c (%) | 7.9±2.0 | 7.6±1.9 | 8.5±2.2 | <0.01 |
| Antidiabetic agents (%) | 158 (72%) | 109 (67%) | 49 (84%) | 0.01 |
| OHA (%) | 109 (50%) | 85 (52%) | 24 (41%) | 0.15 |
| Insulin treatment (%) | 49 (22%) | 24 (15%) | 25 (43%) | <0.01 |
| Retinopathy (%) | 80 (36%) | 33 (20%) | 47 (81%) | <0.01 |
| Non-advanced stage (%) | 42 (19%) | 33 (20%) | 9 (16%) | 0.42 |
| Advanced stage (%) | 38 (17%) | 0 (0%) | 38 (66%) | <0.01 |
| LDL-cholesterol (mg/dL) | 109±32 | 110±31 | 106±34 | 0.47 |
| HDL-cholesterol (mg/dL) | 57±19 | 60±20 | 51±14 | <0.01 |
| Triglycerides (mg/dL) | 108 (81-160) | 102 (70-146) | 120 (86-175) | 0.01 |
| Anti-HL agents (%) | 87 (40%) | 63 (39%) | 24 (41%) | 0.74 |
| Statins (%) | 79 (36%) | 57 (35%) | 22 (38%) | 0.71 |
| Fibrates (%) | 8 (4%) | 6 (4%) | 2 (3%) | 0.93 |
| Cr (mg/dL) | 0.68 (0.59-0.80) | 0.68 (0.57-0.80) | 0.70 (0.61-0.86) | 0.19 |
| eGFR (mL/min/1.73 m$^2$) | 83±24 | 84±22 | 80±28 | 0.57 |
| ACR (mg/g Cr) | 16 (8-86) | 13 (8-34) | 239 (28-300) | <0.01 |
| Nephropathy (%) | 100 (45%) | 55 (34%) | 45 (78%) | <0.01 |
| Non-advanced stage (%) | 70 (32%) | 55 (34%) | 15 (26%) | 0.26 |
| Advanced stage (%) | 30 (14%) | 0 (0%) | 30 (52%) | <0.01 |
| Mean-IMT (mm) | 0.72±0.12 | 0.72±0.12 | 0.73±0.12 | 0.59 |
| Max-IMT (mm) | 1.60±0.84 | 1.48±0.65 | 1.96±1.15 | <0.01 |
| FMD (%) | 2.4±1.9 | 2.5±1.9 | 2.1±1.6 | 0.17 |
| Brachial AWE (kPa) | 721±217 | 694±213 | 797±212 | <0.01 |
| Radial AWE (kPa) | 692±222 | 660±207 | 780±243 | <0.01 |

SBP: systolic blood pressure; DBP: diastolic blood pressure; Anti-HT: antihypertensive; RAS: renin-angiotensin system; CCB: calcium channel blockers; OHA: oral antihyperglycemic agents; LDL: low-density lipoprotein; HDL: high-density lipoprotein; Anti-HL: antihyperlipidemic; Cr: creatinine; eGFR: estimated glomerular filtration rate; ACR: urinary albumin-to-Cr ratio; IMT: intima-media thickness; FMD: flow-mediated vasodilation; AWE: arterial wall elasticity; Pa: Pascal. Microangiopathic complications included advanced-stage retinopathy and nephropathy. The patients were divided into groups with and without microangiopathic complications. The data are presented as the mean±standard deviation, median (interquartile range) or number (%). The levels of triglycerides and Cr and the ACR values were log-transformed due to their skewed distributions. The $p$-values were determined using Student's $t$-test or the chi-square test. Significance level: $p<0.05$.

0.05). Furthermore, there were significant between-group differences in brachial AWE ($p<0.01$) and radial AWE ($p<0.01$), but not in the FMD values ($p=0.65$), after adjusting for antidiabetic medications, antihypertensive medications, antihyperlipidemic medications, retinopathy and nephropathy, in addition to age, sex, the smoking status and the ACR.

The patients' characteristics in the groups with and without microangiopathic complications are shown in **Table 2**. The max-IMT, brachial AWE and

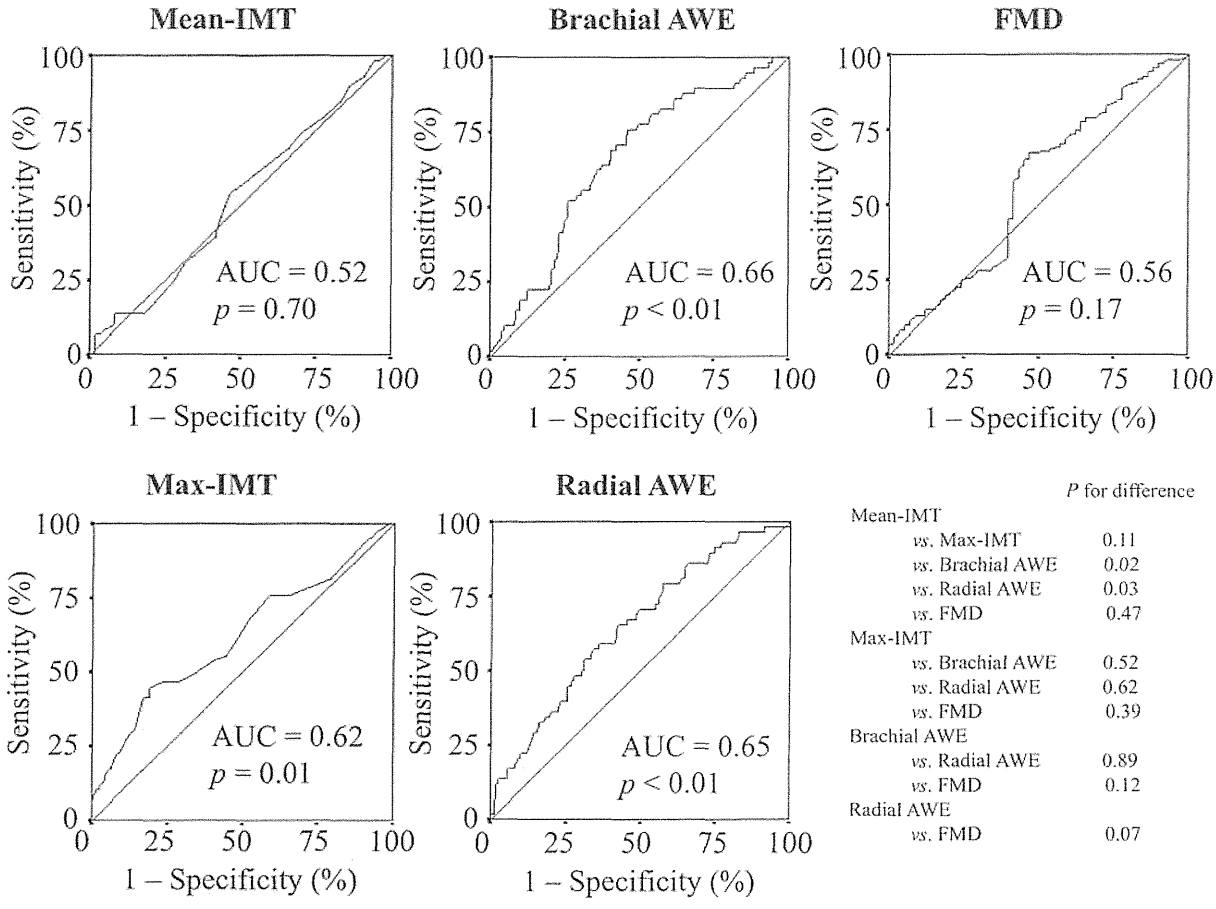


**Fig. 2.** ROC curve analyses of the mean-IMT, max-IMT, brachial AWE, radial AWE and FMD for microangiopathic complications were performed, and the AUCs were measured. ROC: receiver operating characteristic; IMT: intima-media thickness; AWE: arterial wall elasticity; FMD: flow-mediated vasodilation; AUC: area under the receiver operating characteristic curve. Significance level: $p < 0.05$.

radial AWE values were significantly higher in the group with microangiopathic complications than in the group without microangiopathic complications. The ROC curves of the mean-IMT, max-IMT, brachial AWE, radial AWE and FMD regarding microangiopathic complications are shown in **Fig. 2**. The AUCs of the mean-IMT, max-IMT, brachial AWE, radial AWE and FMD were 0.52 (95% confidence interval [CI] 0.43-0.60, $p=0.70$), 0.62 (95% CI 0.53-0.71, $p=0.01$), 0.66 (95% CI 0.58-0.74, $p<0.01$), 0.65 (95% CI 0.57-0.73, $p<0.01$) and 0.56 (95% CI 0.47-0.65, $p=0.17$), respectively. When comparing the AUCs of the mean-IMT, max-IMT, brachial AWE, radial AWE and FMD, no differences were found among the max-IMT, brachial AWE, radial AWE or FMD. The AUCs of the brachial and radial AWE were significantly higher than that of the mean-IMT (**Fig. 2**).

## Discussion

As the number of atherosclerotic risk factors increased, the brachial AWE and radial AWE values increased in the T2DM patients without a history of stroke or coronary artery disease. Although the mean-IMT and max-IMT tended to increase while the FMD decreased, the brachial and radial AWE produced more clear findings of atherosclerotic risk, to some extent, than the other ultrasonic atherosclerosis-related markers. When comparing the groups with and without microangiopathic complications, the max-IMT, brachial AWE and radial AWE values were higher in the group with microangiopathic complications than in the group without microangiopathic complications. Although the AUCs of the brachial and radial AWE did not exhibit a high accuracy, the AUCs of the brachial and radial AWE were approximately equal to that of the max-IMT and higher than

those of the mean-IMT and FMD. Considering that ultrasonic markers for atherosclerosis are not always established[5, 13, 37-40] and the clinical relevance of max-IMT for atherosclerosis has been indicated[41] in T2DM patients, it appears meaningful to note that the brachial and radial AWE may reflect atherosclerotic risks, similar to the max-IMT and superior to the mean-IMT and FMD, in this population.

As atherosclerotic disease progresses, endothelial dysfunction leads to secretory disorders of physiologically active substances, such as nitric oxide derived from the endothelium, inhibition of vascular smooth muscle relaxation and reductions in vascular tone[17, 42, 43]. This can partly induce the proliferation of vascular smooth muscle cells and reduce the vascular smooth muscle function[17, 42, 43]. Histopathological data obtained using the phased tracking method indicate high AWE values in tissue with proliferation of vascular smooth muscle cells[14]. Another study showed that the radial AWE is significantly and positively correlated with the serum C-reactive protein level, an inflammatory index[16]. Inflammation induced by endothelial dysfunction is the primary pathological basis for atherosclerosis[17]. Therefore, AWE measurement can be used to assess the degree of atherosclerotic overload and vascular complications.

FMD is the preferred method for evaluating the endothelial function[34, 35, 44], and the measurements of AWE and FMD are thought to partially overlap. In this study, the FMD tended to decrease in association with an increased number of atherosclerotic risk factors; however, the degree was not large compared with that of brachial and radial AWE. This may be because the patients in our study had poor glycemic control and multiple atherosclerotic risk factors, and the mean FMD value was decreased, even in group 1 (normal range 5-10%[45, 46]).

The carotid artery is a common site of atherosclerosis, and atherosclerotic changes in the carotid artery often reflect systemic atherosclerosis[37, 47, 48]. A large number of studies have demonstrated that the carotid IMT is higher in patients with atherosclerotic risk factors than in healthy subjects[33, 49], while other studies have showed that the carotid IMT is weakly correlated with cardiovascular disease in T2DM patients[13, 38]. Previous studies of the association between the IMT and microangiopathic complications have reported that the mean-IMT is weakly correlated with microangiopathic complications, while the max-IMT is positively correlated with microangiopathic complications[37, 41]. In our study, the carotid IMT did not clearly increase in association with increased atherosclerotic risk factors, and the max-IMT was increased significantly in the patients with microangiopathic complications. Our results support the findings of previous studies[13, 37-39], and we think that the weak correlation observed between the carotid mean-IMT and atherosclerotic risks may be partly due to our study population containing T2DM patients.

The present study is associated with several limitations. The study design was cross-sectional, and cardiovascular event outcomes were not evaluated. Prospective evaluations are needed to confirm the results of our study.

## Conclusions

As the number of atherosclerotic risk factors increased, the brachial and radial AWE values increased in T2DM patients without a history of stroke or coronary artery disease. The brachial and radial AWE used to detect microangiopathic complications were approximately equal to the max-IMT. These results indicate that the brachial and radial AWE can reflect the degree of atherosclerotic overload and may be useful for detecting vascular complications. Measuring the upper limb AWE would be useful for assessing the degree of subclinical atherosclerosis and lead to new approaches for evaluating atherosclerosis.

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## Conflict of Interest Statement

The authors declare that there are no financial or other conflicts of interest.

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ORIGINAL

# Effects of miglitol *versus* sitagliptin on postprandial glucose and lipoprotein metabolism in patients with type 2 diabetes mellitus

Kenta Okada[1)], Hiroaki Yagyu[1)], Kazuhiko Kotani[2)], Hisataka Yamazaki[1)], Kazufumi Ozaki[1)], Manabu Takahashi[1)], Shuichi Nagashima[1)], Jun-ichi Osuga[1)] and Shun Ishibashi[1)]

[1)]*Department of Internal Medicine, Division of Endocrinology and Metabolism, Jichi Medical University, Tochigi 320-0498, Japan*
[2)]*Department of Public Health, Jichi Medical University, Tochigi 320-0498, Japan*

**Abstract.** Postprandial hyperglycemia and/or hyperlipidemia can contribute to development of atherosclerosis in patients with type 2 diabetes mellitus (T2DM). The objective of this study was to compare the effects of miglitol and sitagliptin on postprandial glucose and lipid metabolism in patients with T2DM. Thirty-five patients with T2DM were randomized to 2 groups receiving miglitol (150 mg/day) or sitagliptin (50 mg/day). Serum variables related to glucose and lipid metabolism were measured before and after treatment for 10 weeks and at 0, 60, and 120 min using a cookie-loading test (CLT). After 10 weeks of treatment, miglitol (n = 16) and sitagliptin (n = 18) caused a similarly significant decrease in hemoglobin $A_{1c}$ (mean: 7.6% to 7.3% versus 8.0% to 7.6%) and a significant increase in fasting insulin levels, with a greater increase observed in the miglitol group than in the sitagliptin group ($p$=0.03). In addition, a significant decrease in the change in glucose levels after the CLT was observed in both groups, with a greater decrease observed in the miglitol group than in the sitagliptin group ($p$=0.02). The miglitol group also showed a greater decrease in the change in insulin levels after the CLT than the sitagliptin group ($p$<0.01). The lipid and lipoprotein levels did not show any significant differences between the groups after the CLT. Our results suggested that miglitol and sitagliptin treatment resulted in similar glycemic control but that a greater decrease in postprandial glucose and insulin levels was observed with miglitol compared with sitagliptin in patients with T2DM.

*Key words*: α-Glucosidase inhibitor, Cookie-loading test, Dipeptidyl peptidase-4 inhibitor, Postprandial hyperglycemia, Postprandial hyperlipidemia

**POSTPRANDIAL HYPERGLYCEMIA** and hyperlipidemia have been shown to be risk factors for atherosclerosis [1–3]. Potential agents to target postprandial hyperglycemia and hyperlipidemia in patients with type 2 diabetes mellitus (T2DM) include α-glucosidase inhibitors (AGIs) and dipeptidyl peptidase (DPP)-4 inhibitors. Miglitol is an AGI that improves postprandial hyperglycemia and hyperlipidemia [4] and reduces postprandial insulin requirements without deteriorating lipid profiles [5]. In contrast, sitagliptin improves postprandial hyperglycemia [6] and hyperlipidemia [7, 8]; it has been shown to reduce postprandial lipid levels from both intestinal and hepatic origins, most likely by increasing incretin hormone levels, thereby improving insulin sensitivity and β-cell function [7]. Sitagliptin has been shown to be superior to voglibose, an AGI, in reducing postprandial glucose levels 120 min after consumption of a commercially available meal in which

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Correspondence to: Kenta Okada, M.D., Ph.D., Department of Internal Medicine, Division of Endocrinology and Metabolism, Jichi Medical University, 3311-1 Yakushiji, Shimotsuke-City, Tochigi 329-0498, Japan. E-mail: kokada@jichi.ac.jp

Abbreviations: T2DM, type 2 diabetes mellitus; AGI, α-glucosidase inhibitor; DPP, dipeptidyl peptidase; $HbA_{1c}$, hemoglobin $A_{1c}$; TG, triglyceride; 1,5-AG, 1,5-anhydro-D-glucitol; NEFA, non-esterified fatty acids; ApoB, apolipoprotein B; RLP-C: remnant-like particle cholesterol; CLT, cookie-loading test; SD, standard deviation; ANOVA, analysis of variance; AUC, area under the curve; GLP-1, glucagon-like peptide-1; GIP, glucose-dependent insulinotropic polypeptide.

the total caloric content was approximately 500 kcal, with 75 g carbohydrate and 18.8 g protein [9].

Aoki *et al.* reported the effects of miglitol, sitagliptin, and their combination treatment on plasma glucose, insulin, and incretin levels in healthy men [10]. Each agent reduced postprandial glucose levels within 120 min, and miglitol reduced postprandial insulin levels as well, whereas sitagliptin did not. However, no studies have compared directly postprandial states following treatment with sitagliptin, which enhances insulin levels, and treatment with miglitol, which reduces insulin levels in patients with T2DM. To assess the effects on postprandial hyperglycemia and hyperlipidemia, the aim of the present study was to compare the effects of 10 weeks of treatment with miglitol or sitagliptin on fasting and postprandial glucose and lipid metabolism in patients with T2DM.

## Subjects and Methods

### *Subjects*

Thirty-five patients with T2DM were randomized to 2 groups: one received miglitol (150 mg/day, n=17) and the other received sitagliptin (50 mg/day, n=18) for 10 weeks. Simple randomization was performed in order of clinic visits using a random number table. Based on previous reports that showed a 12-week post-interventional decrease of 0.70% in hemoglobin $A_{1c}$ ($HbA_{1c}$) with sitagliptin [9] and of 0.16% in $HbA_{1c}$ with miglitol [11], the sample size was estimated to be approximately 15 in each group with a significance level ($\alpha$) of 5% and detection level ($1 - \beta$) of 80% (2-tailed). Eligibility criteria were as follows: age > 20 years, the absence of pregnancy, fasting serum triglyceride (TG) levels < 250 mg/dL, $HbA_{1c}$ < 9.4%, serum creatinine level < 1.0 mg/dL, and no changes in medications during the 3 months before enrollment in the study. We also excluded patients with a history of stroke, cardiovascular events, severe microangiopathy, recent acute illness, inflammatory bowel diseases, past history of ileus, type 1 diabetes mellitus, fasting serum C-peptide level < 0.5 ng/mL and patients who were judged as not appropriate for participation. All subjects were instructed not to change their usual dietary habits and continued their medications, such as lipid-lowering drugs including statins and anti-hypertensive drugs, for the duration of the study. The study protocol was approved by the Jichi Medical University Ethical Committee, and written informed consent was obtained from all participants. The protocol for this study was registered with the UMIN Clinical Trial Registry as UMIN000009502, based on the World Medical Association Declaration of Helsinki guidelines.

### *Measurements*

Body mass index was calculated as weight in kilograms/height in square meters. At baseline and after 10 weeks of treatment, blood samples were collected at the outpatient clinic in the morning after overnight fasting for measurement of the following parameters: $HbA_{1c}$, 1,5-anhydro-D-glucitol (1,5-AG), glucose, insulin, glucagon, non-esterified fatty acid (NEFA), total cholesterol, TG, high-density lipoprotein cholesterol, apolipoprotein AI, apolipoprotein AII, apolipoprotein B (ApoB), apolipoprotein CII, apolipoprotein CIII, apolipoprotein E, apolipoprotein B48 (ApoB-48), remnant-like particle cholesterol (RLP-C), aspartate aminotransferase, alanine aminotransferase, and creatinine. A high-performance liquid chromatography (HLC-723 G8; Tosoh, Tokyo, Japan) system was used to measure $HbA_{1c}$ according to the International Federation of Clinical Chemistry reference standard methods [12], and $HbA_{1c}$ values are therefore shown as National Glycohemoglobin Standardization Program equivalent values in this study. 1,5-AG levels were measured using the enzymatic method with the "Lana AG" column-enzyme assay kit (Nippon Kayaku Co. Ltd., Tokyo, Japan) [13]. The homeostasis model assessment of insulin resistance was used to calculate an index from the product of the fasting concentrations of plasma insulin (μIU/mL) and plasma glucose (mg/dL) divided by 405 [14]. Plasma glucagon levels were measured by the radioimmunoassay method using the Glucagon RIA Kit (Merck Millipore, Merck, Ltd., Billerica, MA, USA). Serum NEFA levels were measured using enzyme reagents (NEFA-SS, Eiken Chemical Co. Ltd., Tokyo, Japan) according to standard techniques. Total cholesterol, TG, high-density lipoprotein cholesterol, aspartate aminotransferase, alanine aminotransferase, and creatinine levels were measured by enzymatic methods using an automatic analyzer (Hitachi Co. Ltd., Tokyo, Japan). Low-density lipoprotein cholesterol levels were calculated by the Friedewald equation because all patients had TG levels less than 400 mg/dL [15]. Serum apolipoprotein AI, apolipoprotein AII, ApoB, apolipoprotein CII, apolipoprotein CIII, and apolipoprotein E levels were determined using turbidimetric immunoassays (Apo A-I Auto N "Daiichi," Apo A-II Auto N

"Daiichi," Apo B Auto N "Daiichi," Apo C-II Auto N "Daiichi," Apo C-III Auto N "Daiichi," and Apo E Auto N "Daiichi"; Sekisui Medical Co. Ltd., Tokyo, Japan). ApoB-48 levels were measured by enzyme immunoassay (Lumipulse apo B48; Fujirebio, Inc., Tokyo, Japan) according to the method described by Uchida *et al* [16]. RLP-C levels were measured using an immunoseparation method (RLP-C Jimro II Kit; Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan) [17].

### *Cookie-loading test*

The cookie-loading test (CLT) was performed after an overnight fast using previously described methods [18] at baseline and 10 weeks after the start of each treatment. Subjects were instructed to refrain from smoking and drinking coffee or alcoholic beverages beginning the night before the study. The cookie consisted of 75 g carbohydrate, 28.5 g butter, and 8 g protein for 592 kcal (Saraya Co. Ltd., Osaka, Japan). Subjects were encouraged to ingest the cookie with water within 15 min. Time measurement was started when half of the cookie had been ingested. Sitagliptin and 100 mL water were given 30 min before the CLT as previously reported [9], whereas miglitol and 100 mL water were given immediately before the CLT [19]. Blood samples were obtained during the fasting state before cookie ingestion and at 60 min and 120 min after the cookie load. Plasma glucose, insulin, glucagon, TG, Apo-B, RLP-C, and NEFA levels were determined at each time point.

### *Data analysis*

The primary outcomes were change from baseline in $HbA_{1c}$ at 10 week between the 2 groups. The secondary outcomes were parameters related to glucose metabolism (except $HbA_{1c}$) and lipid metabolism. The data are presented as mean ± standard deviation (SD) for parametric variables, medians with interquartile ranges for nonparametric variables, and numbers for categorical variables. Nonparametric variables were log-transformed in the analyses. Differences between the 2 groups were examined using the unpaired *t*-test and the chi-square test. Differences before and after treatment in each group were examined using paired *t*-tests, and differences before and after treatment between the 2 groups were examined using a 2-way (group and time) analysis of variance (ANOVA). With regard to the effects of the CLT on variables, changes of respective variables within each group were examined using repeated-measures ANOVA (followed by *post-hoc* multi-comparison Bonferroni tests), and changes between the 2 groups were examined using 2-way (group and timing) ANOVA. Correlations between changes in variables were examined using Pearson tests. Changes in respective variables after the CLT were also assessed by the area under the curve (AUC), calculated using the trapezoidal method [20]. Statistical significance was set at a *p*-value of less than 0.05. All analyses were performed using the SPSS II software package (IBM Corporation, Armonk, NY, USA).

## Results

### *Patients*

After randomization, one patient in the miglitol group dropped out of the study because of abdominal distention. The demographic characteristics of the patients were similar between the 2 treatment groups (Table 1), and variables related to glucose and lipid metabolism were similar between the 2 groups. However, the sitagliptin group had significantly higher levels of fasting insulin and homeostasis model assessment of insulin resistance than the miglitol group (Table 2).

### *Parameters related to glucose metabolism*

The changes from baseline in $HbA_{1c}$, as the pri-

**Table 1** Patient characteristics

| | Miglitol | Sitagliptin | *p*-value |
|---|---|---|---|
| Men/women | 8/8 | 5/13 | 0.18 |
| Age (years) | 64.9 ± 10.2 | 66.3 ± 6.7 | 0.65 |
| Duration of diabetes (years) | 7.0 (4.3-14.3) | 9.5 (5.5-15.3) | 0.35 |
| Use of antidiabetic agent, % (n) | | | |
| Diet only | 31.3 (5) | 11.1 (2) | 0.15 |
| Sulfonylurea | 56.3 (9) | 66.7 (12) | 0.82 |

Data are presented as the number, percentage, mean ± SD, or median (interquartile range). $p<0.05$ as a comparison between the miglitol and sitagliptin groups.

**Table 2** Clinical data for each variable at baseline and after 10 weeks of sitagliptin or miglitol treatment in patients with type 2 diabetes mellitus

| | Miglitol | | | Sitagliptin | | | | |
|---|---|---|---|---|---|---|---|---|
| Parameters | Baseline | Week 10 | $p^1$ | Baseline | Week 10 | $p^1$ | $p^2$ | $p^3$ |
| Body mass index (kg/m$^2$) | 23.7 ± 2.9 | 23.7 ± 2.9 | 0.81 | 25.0 ± 3.9 | 25.0 ± 3.9 | 0.92 | 0.29 | 0.28 |
| $HbA_{1c}$ (%) | 7.6 ± 0.6 | 7.3 ± 0.7 | < 0.01* | 8.0 ± 0.7 | 7.6 ± 0.6 | < 0.01* | 0.10 | 0.23 |
| 1,5-AG (μg/mL) | 7.2 (4.0-11.6) | 12.9 (7.4-18.5) | < 0.01* | 6.0 (3.6-10.0) | 10.2 (6.9-16.2) | < 0.01* | 0.53 | 0.43 |
| Glucose (mg/dL) | 131 ± 26 | 138 ± 26 | 0.21 | 142 ± 27 | 129 ± 22 | 0.06 | 0.26 | 0.90 |
| Insulin (μIU/mL) | 3.8 (2.5-5.0) | 5.8 (2.8-7.2) | 0.11 | 5.4 (4.5-7.0) | 6.3 (4.0-8.9) | 0.15 | 0.03* | 0.03* |
| HOMA-IR | 1.16 (0.79-2.01) | 1.71 (0.85-2.62) | 0.10 | 2.08 (1.45-2.38) | 2.08 (1.29-3.02) | 0.89 | 0.03* | 0.05 |
| Glucagon (pg/mL) | 65.5 ± 21.8 | 67.4 ± 24.6 | 0.60 | 68.8 ± 17.8 | 67.9 ± 21.1 | 0.86 | 0.63 | 0.78 |
| NEFA (μEq/L) | 651 (339-651) | 511 (375-768) | 0.69 | 636 (495-793) | 647 (473-714) | 0.26 | 0.40 | 0.37 |
| Total cholesterol (mg/dL) | 198 ± 28 | 199 ± 29 | 0.91 | 186 ± 35 | 181 ± 28 | 0.42 | 0.28 | 0.13 |
| Triglyceride (mg/dL) | 121 (78-236) | 97 (80-167) | 0.21 | 109 (67-174) | 122 (81-135) | 0.87 | 0.35 | 0.43 |
| HDL-cholesterol (mg/dL) | 58 ± 14 | 58 ± 13 | 0.86 | 60 ± 18 | 57 ± 16 | 0.14 | 0.74 | 0.96 |
| LDL-cholesterol (mg/dL) | 108.3 ± 25.3 | 114.8 ± 25.3 | 0.24 | 99.6 ± 26.3 | 100.0 ± 22.6 | 0.94 | 0.34 | 0.14 |
| Apolipoprotein A I (mg/dL) | 147.1 ± 27.6 | 141.8 ± 25.0 | 0.03* | 154.3 ± 28.7 | 150.3 ± 27.9 | 0.32 | 0.46 | 0.39 |
| Apolipoprotein A II (mg/dL) | 30.5 ± 8.2 | 28.0 ± 8.0 | < 0.01* | 31.8 ± 4.8 | 31.3 ± 4.5 | 0.53 | 0.58 | 0.30 |
| Apolipoprotein B (mg/dL) | 102.3 ± 15.5 | 102.8 ± 19.8 | 0.87 | 98.1 ± 18.1 | 94.4 ± 16.1 | 0.46 | 0.08 | 0.10 |
| Apolipoprotein C II (mg/dL) | 5.3 ± 2.7 | 5.4 ± 3.0 | 0.83 | 3.8 ± 1.6 | 3.8 ± 1.4 | 0.78 | 0.05 | 0.05 |
| Apolipoprotein C III (mg/dL) | 10.6 ± 4.3 | 9.7 ± 3.7 | 0.04* | 9.7 ± 3.3 | 9.4 ± 2.6 | 0.59 | 0.50 | 0.63 |
| Apolipoprotein E (mg/dL) | 4.8 ± 0.9 | 4.5 ± 0.9 | 0.19 | 4.2 ± 1.3 | 4.3 ± 1.3 | 0.30 | 0.13 | 0.28 |
| Apolipoprotein B48 (μg/mL) | 2.9 (1.7-6.7) | 2.9 (2.2-6.8) | 0.88 | 4.3 (2.1-5.8) | 3.3 (1.8-5.3) | 0.01* | 0.69 | 0.97 |
| RLP-C (mg/dL) | 4.1 (3.0-7.9) | 3.4 (2.6-5.2) | 0.10 | 4.0 (2.7-5.2) | 3.6 (3.3-4.8) | 0.81 | 0.39 | 0.62 |
| AST (IU/L) | 21 (17-28) | 20 (17-26) | 0.55 | 19 (18-27) | 23 (18-33) | 0.19 | 0.51 | 0.27 |
| ALT (IU/L) | 20 (16-32) | 24 (16-30) | 0.82 | 21 (15-24) | 26 (16-32) | 0.07 | 0.86 | 0.51 |
| Creatinine (mg/dL) | 0.69 ± 0.18 | 0.70 ± 0.19 | 0.50 | 0.60 ± 0.10 | 0.61 ± 0.12 | 0.54 | 0.06 | 0.07 |

$HbA_{1c}$, hemoglobin $A_{1c}$; 1,5-AG, 1,5-anhydro-D-glucitol; HOMA-IR, homeostasis model assessment of insulin resistance; NEFA, non-esterified fatty acid; HDL, high-density lipoprotein; LDL, low-density lipoprotein; RLP-C, remnant-like particle cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase. Data are presented as the mean ± SD or median (interquartile range). *$p^1$<0.05, comparison of respective data between baseline and after 10 weeks of treatment with miglitol or sitagliptin; *$p^2$<0.05, comparison of baseline data between the miglitol and sitagliptin groups; *$p^3$<0.05, comparison of changes in respective data between the miglitol and sitagliptin groups; When the patient in the miglitol group who dropped out was considered in the analyses, the results ($p$-values) did not change.

mary endpoint, at 10 week were similar between the 2 groups (Table 2). After 10 weeks of treatment, $HbA_{1c}$ levels were significantly decreased in both groups, whereas 1,5-AG levels were significantly increased in both groups. Treatment with miglitol significantly increased changes in fasting insulin levels compared with treatment with sitagliptin at 10 weeks ($p$=0.03).

Plasma glucose, insulin, and glucagon levels during the CLT before and after treatment are shown in Fig. 1. Plasma glucose, insulin, and glucagon levels were significantly increased during the CLT before and after 10 weeks for both treatments. After 10 weeks of treatment, both the miglitol and sitagliptin groups exhibited a significant reduction in glucose levels before and at 60 and 120 min after the CLT. Mean changes in decreased glucose levels during the CLT were significantly greater in the miglitol group than in the sitagliptin group ($p$=0.02). The changes in insulin and glucagon levels were different between the miglitol and sitagliptin groups. After 10 weeks of treatment, plasma insulin levels were significantly increased in both groups after the CLT. However, changes in insulin levels were significantly increased in the sitagliptin group but were suppressed in the miglitol group ($p$<0.01). Plasma glucagon levels were significantly increased in the miglitol group after the CLT. In contrast, plasma glucagon levels remained essentially stable in the sitagliptin group after the CLT.

The AUCs for glucose, insulin, and glucagon levels during the CLT before and after treatment are shown

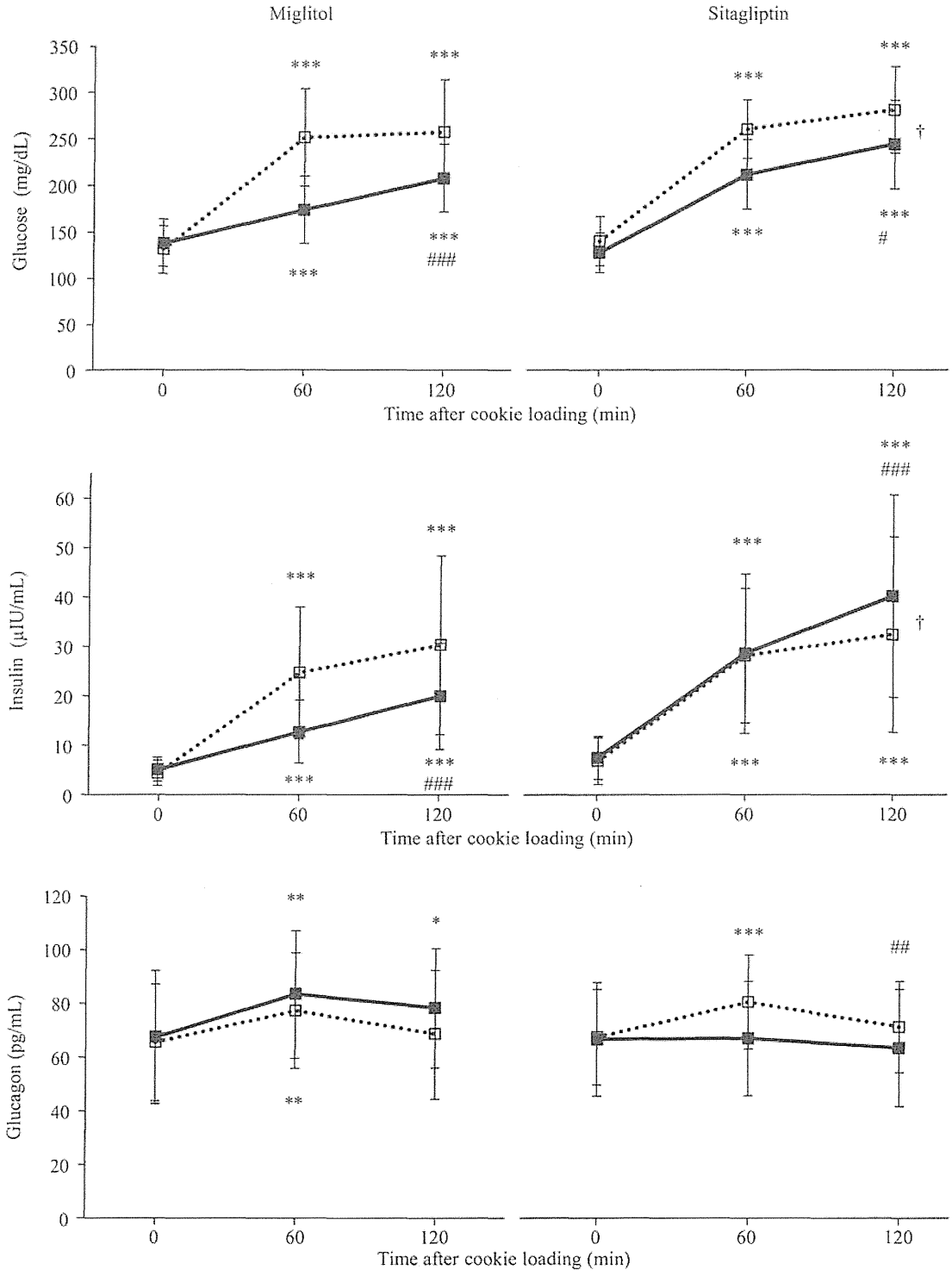


**Fig. 1** Time course of glucose, insulin, and glucagon levels after the cookie-loading test at baseline and after 10 weeks of treatment with sitagliptin or miglitol in patients with type 2 diabetes mellitus. Data are presented as the mean ± standard deviation. Two-way analysis of variance was followed by a Bonferroni *post hoc* test. Open squares (□), at baseline; Closed squares (■), at 10 weeks. ***$p<0.01$ *versus* time 0 min, *$p<0.05$ *versus* time 0 min, **$p=0.01$ *versus* time 0 min, ###$p<0.01$ *versus* time 60 min, #$p<0.05$ *versus* time 60 min, ##$p=0.01$ *versus* time 60 min. Comparison of repeated changes in respective data at baseline or after 10 weeks of treatment with miglitol or sitagliptin. †$p<0.05$ *versus* Miglitol.

**Table 3** Changes in each AUC variable at baseline and after 10 weeks of treatment with sitagliptin or miglitol in patients with type 2 diabetes mellitus

| Parameters | Time (min) | Miglitol | | $p^1$ | Sitagliptin | | $p^1$ | $p^2$ |
|---|---|---|---|---|---|---|---|---|
| | | Baseline | Week 10 | | Baseline | Week 10 | | |
| Glucose (mg min/dL) | 0-60 | 11481 ± 2075 | 9334 ± 1710 | < 0.01* | 12172 ± 1578 | 10367 ± 1509 | < 0.01* | 0.11 |
| | 60-120 | 15259 ± 2907 | 11424 ± 1973 | < 0.01* | 16461 ± 2105 | 13858 ± 2413 | < 0.01* | 0.02*,† |
| | 0-120 | 26739 ± 4890 | 20758 ± 3599 | < 0.01* | 28633 ± 3420 | 24165 ± 3804 | < 0.01* | 0.03*,† |
| Insulin (μIU min/dL) | 0-60 | 783 (500-1084) | 475 (356-671) | < 0.01* | 963 (970-1309) | 944 (671-1382) | 0.68 | < 0.01* |
| | 60-120 | 1544 (971-2110) | 900 (642-1315) | < 0.01* | 1994 (1204-2481) | 2651 (1735-3565) | 0.09 | 0.02* |
| | 0-120 | 2400 (1487-3233) | 1444 (1028-1962) | < 0.01* | 2651 (1735-3565) | 2818 (1814-3780) | 0.20 | 0.01* |
| Glucagon (pg min/dL) | 0-60 | 4280.6 ± 1205.8 | 4522.5 ± 1370.5 | 0.16 | 4518.3 ± 943.1 | 4086.7 ± 1230.0 | 0.10 | 0.80 |
| | 60-120 | 4370.6 ± 1313.8 | 4846.9 ± 1318.5 | < 0.01* | 4630.0 ± 999.0 | 3988.3 ± 1246.4 | 0.02* | 0.45 |
| | 0-120 | 8651.3 ± 2471.5 | 9369.4 ± 2638.2 | 0.01* | 9148.3 ±1912.6 | 8075.0 ± 2453.9 | 0.04* | 0.61 |
| Triglyceride (mg min/dL) | 0-60 | 7605 (4958-13260) | 5250 (4815-9735) | 0.01* | 7580 (4958-9735) | 8025 (6255-8723) | 0.65 | 0.78 |
| | 60-120 | 9000 (6060-15007) | 7065 (5152-10380) | < 0.01* | 9915 (6870-12570) | 9960 (8032-11828) | 0.96 | 0.97 |
| | 0-120 | 16605 (10560-28125) | 12465 (9968-20115) | < 0.01* | 17895 (12300-21990) | 17805 (14940-20415) | 0.83 | 0.91 |
| Apolipoprotein B48 (μg min/mL) | 0-60 | 269 (195-548) | 201 (150-450) | < 0.01* | 333 (248-529) | 270 (191-386) | < 0.01* | 0.53 |
| | 60-120 | 401 (290-769) | 348 (239-581) | 0.01* | 464 (346-617) | 381 (316-521) | 0.03* | 0.65 |
| | 0-120 | 669 (498-1317) | 552 (423-1031) | < 0.01* | 805 (596-1186) | 651 (522-910) | 0.01* | 0.60 |
| RLP-C (mg min/dL) | 0-60 | 287 (212-530) | 228 (181-342) | < 0.01* | 320 (209-386) | 264 (225-336) | 0.14 | 0.99 |
| | 60-120 | 369 (266-608) | 320 (200-426) | < 0.01* | 419 (302-504) | 353 (278-410) | 0.11 | 0.97 |
| | 0-120 | 656 (483-1136) | 555 (383-789) | < 0.01* | 746 (500-896) | 611 (500-731) | 0.12 | 0.98 |
| NEFA (μEq min/L) | 0-60 | 29762 ± 1081 | 28633 ± 1093 | 0.71 | 31237 ± 7411 | 29700 ± 9155 | 0.42 | 0.49 |
| | 60-120 | 22866 ± 6308 | 22929± 8131 | 0.97 | 21712 ± 4617 | 21563 ± 7488 | 0.91 | 0.74 |
| | 0-120 | 52629 ± 16215 | 51563 ± 18353 | 0.81 | 52948 ± 10844 | 51260 ± 16410 | 0.58 | 0.84 |

RLP, remnant-like particle cholesterol; NEFA, non-esterified fatty acid. Data are presented as the mean ± SD or median (interquartile range). $p^1$, comparison of respective data between baseline and after 10 weeks of treatment with miglitol or sitagliptin; $p^2$, comparison of changes in respective data between the miglitol and sitagliptin groups. †When baseline insulin-adjusted analysis was performed, the results ($p$-values) did not change.

in Table 3. Before treatment, the AUCs for glucose and insulin levels were similar between the 2 groups. After 10 weeks of treatment, the AUC for glucose levels was significantly decreased in both groups ($p<0.01$ in both groups). In particular, the AUC for glucose levels decreased significantly from 60 to 120 min in the miglitol group but not in the sitagliptin group ($p=0.02$). After 10 weeks of treatment, the AUC for insulin levels was significantly decreased in the miglitol group. On the other hand, the AUC for insulin levels tended to increase in the sitagliptin group. Although the AUC for glucagon levels was increased in the miglitol group, especially from 60 to 120 min, the AUC for glucagon levels was significantly decreased in the sitagliptin group. In addition, although the patient in the miglitol group who dropped out was considered in these analyses, the results did not change significantly. When an at-baseline insulin adjustment was performed in the analyses of changes in glucose levels after CLT, the results did not change (data not shown).

***Parameters related to lipid metabolism***

Plasma TG, ApoB-48, and RLP-C levels were significantly increased and NEFA levels were significantly decreased during the CLT before and after 10 weeks of treatment for both groups (Table 4). However, TG, ApoB-48, RLP-C, and NEFA levels did not significantly change in both groups at 10 weeks after the CLT as compared with week 0.

The miglitol group exhibited a significant reduction in the AUCs of TG, ApoB-48, and RLP-C after 10 weeks of treatment (Table 3). On the other hand, the sitagliptin group exhibited a significant reduction only in the AUC of ApoB-48 after 10 weeks of treatment. The AUCs for NEFA were similar in both groups before and after 10 weeks for each treatment.

**Table 4** Changes in postprandial levels of each variable at baseline and after 10 weeks of treatment with sitagliptin or miglitol in patients with type 2 diabetes mellitus

| Parameters | Time (min) | Miglitol | | | | | Sitagliptin | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 60 | 120 | $p^1$ | $p^2$ | 0 | 60 | 120 | $p^1$ | $p^2$ | $p^3$ |
| Triglyceride (mg/dL) | Baseline | 121 (77-214) | 133 (84-228)[a] | 168 (115-276)[a,c] | < 0.01* | | 116 (66-150) | 151 (101-177)[a] | 185 (125-249)[a,c] | < 0.01* | | 0.42 |
| | Week 10 | 92 (80-163) | 93 (80-162) | 138 (95-187)[a,c] | < 0.01* | 0.28 | 122 (102-137) | 146 (115-160)[a] | 189 (149-227)[a,c] | < 0.01* | 0.87 | 0.61 |
| Apolipoprotein B48 (μg/mL) | Baseline | 2.9 (1.7-6.7) | 6.2 (5.0-11.6)[a] | 7.3 (5.0-14.3)[a] | < 0.01* | | 4.3 (2.1-5.8) | 7.2 (6.1-10.6)[a] | 8.3 (5.5-10.4)[a,c] | < 0.01* | | 0.78 |
| | Week 10 | 2.9 (2.2-6.8) | 4.2 (2.8-8.2)[b] | 8.1 (4.2-11.2)[a,c] | < 0.01* | 0.43 | 3.3 (1.8-5.3) | 6.0 (4.5-7.8)[a] | 7.3 (5.3-9.4)[a,d] | < 0.01* | 0.22 | 0.74 |
| RLP-C (mg/dL) | Baseline | 4.1 (3.0-7.9) | 5.5 (4.4-9.8)[a] | 6.8 (4.8-10.6)[a,c] | < 0.01* | | 4.0 (2.7-5.2)[a] | 6.6 (4.5-7.6)[a] | 7.1 (5.2-9.3)[a] | < 0.01* | | 0.60 |
| | Week 10 | 3.4 (2.6-5.2) | 4.3 (3.2-6.2) | 6.5 (3.8-8.0)[a,c] | < 0.01* | 0.25 | 3.6 (3.2-4.9) | 5.0 (4.3-6.2)[a] | 6.7 (5.0-7.4)[a,c] | < 0.01* | 0.46 | 0.74 |
| NEFA (μEq/L) | Baseline | 651 (339-761) | 367 (328-439)[a] | 394 (334-430)[a,c] | < 0.01* | | 636 (495-793) | 371 (320-484)[a] | 307 (271-385)[a,d] | < 0.01* | | 0.85 |
| | Week 10 | 511 (375-768) | 339 (242-556)[a] | 385 (270-466)[a,c] | < 0.01* | 0.81 | 647 (473-714) | 405 (304-491)[a] | 287 (240-389)[a,c] | < 0.01* | 0.51 | 0.93 |

RLP-C, remnant-like particle cholesterol; NEFA, non-esterified fatty acid. Data are presented as the median (interquartile range). $p^1$, comparison of repeated changes in respective data at baseline or after 10 weeks of treatment with miglitol or sitagliptin; $p^2$, comparison of repeated changes in respective data between baseline and after 10 weeks treatment with miglitol or sitagliptin; $p^3$, comparison of repeated changes in respective data between the miglitol and sitagliptin groups at baseline or after 10 weeks of treatment. [a]$p<0.01$ *versus* time 0 min, [b]$p<0.05$ *versus* time 0 min, [c]$p<0.01$ *versus* time 60 min, [d]$p<0.05$ *versus* time 60 min. When the patient in the miglitol group who dropped out was considered in the analyses, the results ($p$-values) did not change.

## Discussion

Although both miglitol and sitagliptin treatment similarly reduced glucose levels after 10 weeks, miglitol caused a greater reduction in postprandial hyperglycemia through a mechanism involving the regulation of insulin levels that was contradictory to that of the sitagliptin mechanism. This head-to-head prospective comparative study between miglitol and sitagliptin treatment for 10 weeks revealed different changes in insulin and glucagon levels during the CLT.

Elevation of postprandial glucose levels was suppressed significantly by both miglitol and sitagliptin in this study. However, postprandial insulin and glucagon levels were different between the 2 groups. Lower plasma insulin levels with higher glucagon levels were seen in the miglitol group, whereas a moderate increase in insulin levels with lower glucagon levels was seen in the sitagliptin group. Miglitol enhances glucagon-like peptide-1 (GLP-1) secretion and reduces glucose-dependent insulinotropic polypeptide (GIP) secretion through absorption in the upper portion of the small intestine; therefore, a relatively higher amount of carbohydrates was absorbed in the lower portion of the small intestine [5, 10, 21, 22]. The tendency for decreased insulin levels may explain the increased glucagon levels with miglitol treatment [23]. In contrast, sitagliptin has been shown to enhance the activity of GLP-1 and GIP by inhibiting DPP-4 enzymatic activity; therefore, sitagliptin increases serum insulin and decreases serum glucagon levels in patients with T2DM [6, 24]. A meal tolerance test such as the CLT has a greater effect on GIP secretion than GLP-1 secretion, and the GIP level peaked at 30 min in Japanese patients with T2DM [25]. GIP contributes more to secretion of insulin than does GLP-1 [26] on the basis of the baseline $HbA_{1c}$ in this study. Thus, although we did not measure GLP-1 and GIP levels as well as glucose and lipid parameters at 30 min during the CLT in this study, we expect that this difference can be partly explained by the fact that glucagon, in addition to GIP rather than GLP-1, is regulated by insulin levels [27].

In our study, mean values of changing postprandial hyperlipidemia exhibited similar reductions in