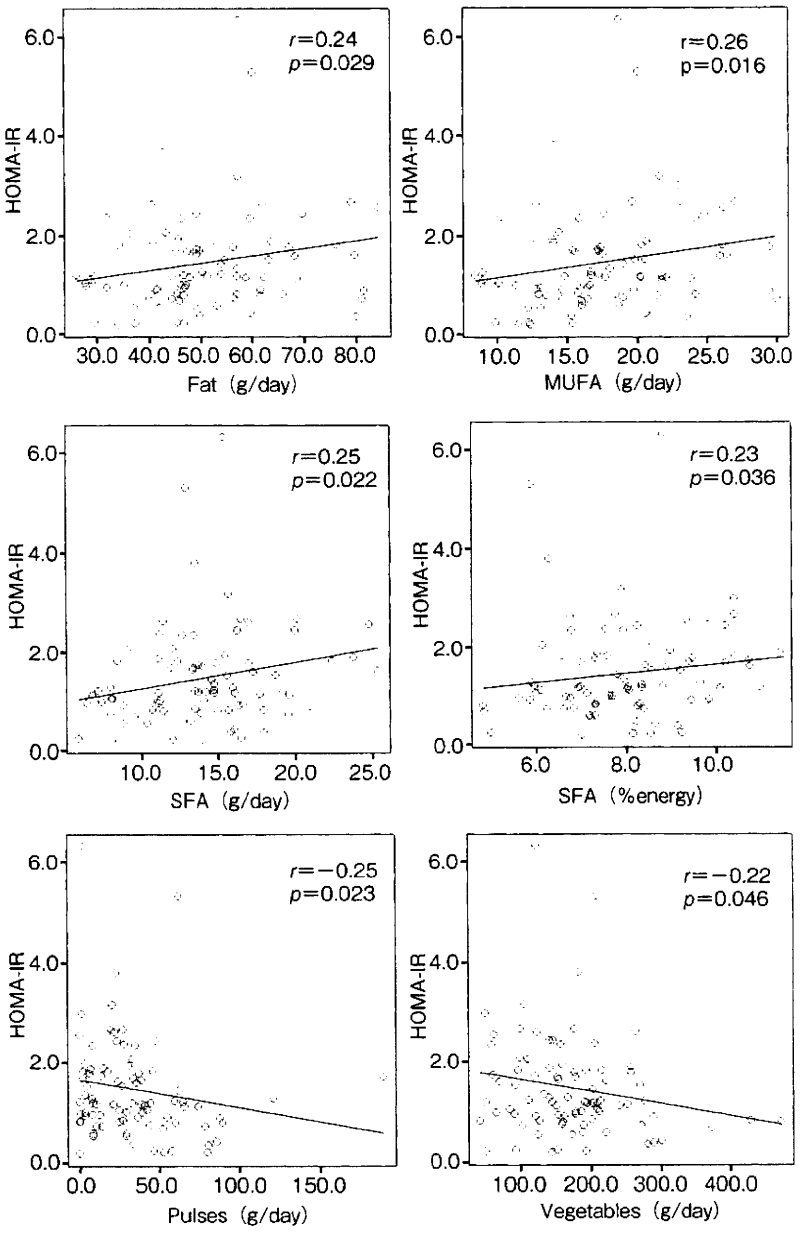

Fig. 1 Correlation between HOMA-IR and dietary factors in young Japanese women

60%). βカロテン当量は野菜類($r=0.70$, $p<0.01$), 調味料類($r=0.50$, $p<0.01$), 藻類($r=0.36$, $p<0.01$)と相関した. βカロテン当量を目的変数とした多変量解析では, 野菜類(正)がβカロテン当量の最大の規定因子であった(寄与率56%). このほか, 豆類(正), 種実類(負)が規定因子にみられた(寄与率60%).

考 察

本研究は若年女性におけるインスリン抵抗性について食事因子を中心に, 食事調査から得られた摂取栄養素, 摂取食品群のデータを用いて詳細な検討を行った. 栄養素との関連では, HOMA-IRは脂質, SFA, MUFAの1日摂取量, SFAエネルギー比と正相関した. インスリン抵抗性はアディポサイトカインとの関連性も示されており, なかでもレプチン, PAI-1, アディポネクチンはインスリン抵抗性と関連深い[9]. 多変量解析では, 体脂肪量とこれらのアディポサイトカインとは独立してHOMA-IRを規定する因子としてSFA(正)と野菜類(負)が認められた. 栄養素における多変量解析ではSFA以外にβカロテン当量(負)が, 食品群における多変量解析では野菜類以外に乳類(正)も規定因子に認められた.

本研究では, SFAは乳類, 次いで肉類と正相関を認めた. 乳類は酪酸, ミリスチン酸, パルミチン酸,

Table 4 Anthropometric, biochemical, and dietary parameters stratified by insulin resistance after adjustment for body fat percentage

	Normal (n = 56)	Median (n = 17)	High (n = 12)
HOMA-IR	0.9±0.4	1.9±0.2	3.3±1.2
Body fat percent (%)	28.5±4.8	29.4±5.0	33.6±6.9
BMI (kg/m^2)	20.4±0.2	20.1±0.4	21.6±0.5[a]
Fasting plasma glucose (mg/d*l*)	83.0±0.8	89.7±1.5[b]	92.4±1.9[c]
Fasting plasma insulin (μU/m*l*)	4.7±0.3	8.8±0.5[c]	14.2±0.7[c]
Plasminogen activator inhibitor-1 (ng/m*l*)	16.5±1.0	16.1±1.8	23.8±2.2[a]
Energy (kcal)	1,533±43	1,478±78	1,859±97[a]
Carbohydrate (g)	210.6±6.4	202.3±11.5	254.5±14.3[a]
Fatty acid (g)	49.2±1.8	49.7±3.3	62.6±4.1[a]
Saturated (g)	13.2±0.5	13.9±1.0	17.8±1.2[b]
Monounsaturated (g)	16.8±0.7	17.5±1.2	21.5±1.5[a]
Saturated (% energy)	7.7±0.2	8.4±0.3	8.8±0.4[a]
Grain (g)	326.1±13.4	303.9±24.1	427.4±30.1[a]
Milk · dairy products (g)	140.1±13.1	132.6±23.6	220.3±29.4[a]

Mean±SE. Normal insulin resistance was defined as HOMA-IR<1.6 and high as ≧2.5, based on criteria in the 2007 Treatment Guide for Diabetes edited by the Japan Diabetes Society. a : $p<0.05$, b : $p<0.01$, c : $p<0.001$ versus Normal group

Table 5 Stepwise multiple logistic regression analysis for HOMA-IR

Model	r^2	β	SE (β)	p value
1	0.26			0.000
Body fat mass (kg)		0.0001	0.000	0.000
Saturated fatty acid (g)		0.065	0.023	0.006
Vegetables (g)		−0.003	0.001	0.020
2	0.25			0.000
Body fat mass (kg)		0.0001	0.000	0.000
Saturated fatty acid (g)		0.066	0.023	0.005
Beta carotene potency (μg)		−0.0002	0.000	0.023
3	0.27			0.000
Body fat mass (kg)		0.0001	0.000	0.000
Milk and/or dairy products (g)		0.003	0.001	0.003
Vegetables (g)		−0.003	0.001	0.007

Body fat mass measured using DXA, plasma levels of leptin, plasminogen activator inhibitor-1, and adiponectin were included in all models. In addition to the 3 variables, model 1 included all nutrients and all food, model 2 included all nutrients, and model 3 included all food

ステアリン酸等のSFAを多く含み，脂肪酸の中でもSFAの割合が比較的高い食品群である[10]．一般にSFAと言えば肉類が想像されることが多いが，SFAの供給源として鶏卵，牛乳，精白米が上位を占めるとの報告もある[11]．乳類は若年女性の嗜好に合った，手軽に摂取し易い食品の1つと考えられるが，本研究の対象では国民栄養調査における同年代の女性に比べ，乳類の平均摂取量は1.5倍であった．インスリン抵抗性とSFAとの関係に関する報告として，中高年男性におけるSFA摂取の増加は空腹時インスリン濃度の上昇と関連したとの報告がある[12]．SFA食とMUFA食の割付け研究では，健康な中年男女においてMUFA食でインスリン感受性は変化しなかったが，SFA食でインスリン感受性が低下し，また，総脂質摂取量を抑えたうえでMUFA摂取を増すことの有益性が示されている[13]．このほか，高SFA食はインスリン抵抗性を惹起し，脂質摂取がそう過大でない範囲において(脂質エネルギー比37%未満)，高MUFA

食のインスリン感受性への有益性も報告されている[14]．これらの報告からも，インスリン抵抗性の抑制においては，脂質の摂取量を減らし脂質エネルギー比を下げ，かつ脂肪酸組成(脂肪酸の種類とその割合)も重要であり，SFAの摂取割合を下げることが重要である．本研究でもHOMA-IRとSFAとの関係において量，エネルギー比ともに正の関係が認められ，HOMA-IR 2.5以上のインスリン抵抗性群においてSFAの量，SFAエネルギー比ともに高値が認められた．したがって，若年女性においてもインスリン抵抗性の増大を防ぐためには，SFAの摂取の過剰に注意を払う必要があり，その摂取として乳類の過剰摂取に注意する必要があると思われる．なお，MUFAについて，本研究ではHOMA-IR 2.5以上の対象において体脂肪率調整後のMUFA摂取量が多いなど，HOMA-IRと正の関係を示唆する結果もみられたが，MUFAの効果は前述のように脂質の摂取を抑えた上での有益性が報告されている．本研究では平均脂質エネルギー比が29.2%と，18～29歳女性の目標量(20%以上30%未満)[8]の上限値であった．また，脂質エネルギー比30%以上が，対象の44.7%に認められた．インスリン抵抗性の観点からみた日本人，若年女性における脂質エネルギー比の問題やMUFAの影響については，今後の検討が必要であると考える．

本研究では，若年女性において，HOMA-IRと野菜類との間に負の相関を認めた．先行研究では，アメリカ人女性(BMI 29未満)において，果物の摂取は糖尿病の発症リスクと関係しなかったが，野菜類の摂取と負の関係を認めた報告[15]や，カロテノイドを多く含む野菜類や果物の摂取は糖尿病のハイリスク集団に対して予防的な役割をもつ報告がある[16]．また，本研究では，HOMA-IRはβカロテン当量と負の相関を認めた．今回のβカロテン当量は，βカロテンとαカロテンおよびクリプトキサンチンのβカロテン当量を合計したものである[6]．2型糖尿病とカロテノイドとの関係について，先行研究では，フィンランドの中高年男女において，カロテノイド，その中でもβクリプトキサンチンの摂取が2型糖尿病の発症リスクの減少と相関したとの報告がある[17]．フィンランドでのケースコントロールスタディでは，血清αトコフェロールおよびβカロテンが高い者において，2型糖尿病の発症リスクが減少していた報告がある[18]．日本においては，健康診断受診の中年男女において，HOMA-IRで評価したインスリン抵抗性と血清カロテノイドとの間に負の相関が認められている[19]．

以上の所見は，非肥満の若年女性でみられたHOMA-IRと野菜類およびβ-カロテン当量との間における関係と一致するものと思われる．さらに本研究では，野菜類がβカロテン当量の最大の供給源であり，野菜類の摂取の意義が高いことが示唆された．

カロテノイドのほか，食物成分で抗酸化作用を有し，2型糖尿病のリスク軽減に関係する成分としてビタミンE(トコフェロール)やビタミンCの報告があるが[17,20]，本研究ではビタミンE(αトコフェロール当量)およびビタミンCの摂取量と，インスリン抵抗性との間に有意な相関はみられなかった(順に$r=0.08$，$r=-0.05$)．また，今回は各種トコフェロール別(α，β，γ，δトコフェロール)や抗酸化作用を持つポリフェノール等については未検討であり，インスリン抵抗性を軽減する食物成分としてβカロテン以外の関与成分の可能性も考慮に入れるべきである．

本研究では，食事調査の手法として秤量食事記録(食物記録法)を用いた．この手法は食物摂取頻度調査の妥当性を検証するためのゴールドスタンダードとして推奨され[21]，国民栄養調査にも用いられている．しかし，国民栄養調査の記録は1日のみである．本研究ではすべての曜日を含んだ7日間を詳細に調査した．一方，秤量食事記録の限界として，被験者の記録に対する負担感から食事内容の簡素化，過少報告等の可能性は否定できない[22]．また，今回インスリン抵抗性の指標に用いたHOMA-IRはグルコースクランプ法やミニマムモデルによって求められたインスリン感受性指数と有意に相関し，日常臨床および大規模研究においてもインスリン抵抗性を簡易に評価しうる非侵襲性の指標として広く用いられている[23]．しかし，インスリン抵抗性が過大または過小評価されることもあり[24～26]，HOMA-IRがインスリン抵抗性の絶対的指標ではないことも考慮に入れておく必要がある．

本研究では，平均BMIが20.5 kg/m^{2}の若年女性においても，体脂肪量はHOMA-IRを規定する独立因子であった．したがって，若年女性においてもインスリン抵抗性の増大を予防するには体脂肪のこれ以上の蓄積を予防することが重要であると思われる．食事では，乳類も考慮に入れたSFAの過剰摂取に注意を払い，βカロテンの補給をふまえ，野菜類の積極的な摂取を含んだ食事療法が推奨される．

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Abstract

Saturated Fat and Vegetable Intake Associated with Insulin Resistance in Young Japanese Women

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To determine whether diet is associated with obesity-independent insulin resistance, we studied how dietary content and caloric intake relate to insulin resistance in 85 women college students aged 18-22 years. Subjects were weighed daily during the seven-consecutive-day dietary assessment. Insulin resistance was measured using homeostasis model assessment (HOMA-IR) and body composition was determined by dual-energy X-ray absorptiometry (DXA). Twelve (14%) of the 85 had HOMA-IR of ≦2.5 and consumed more energy, carbohydrates, protein, and fat-specifically, saturated fat-than did those with HOMA-IR of <1.6. In stepwise multiple regression analysis including all nutrients as independent variables, body fat mass, saturated fat, and beta carotene potency (inverse) were significant determinants of HOMA-IR independent of leptin, adiponectin, and plasminogen activator inhibitor-1. Dairy products and vegetables (inverse) were significant independent predictors of HOMA-IR in multiple regression analysis including all foods as independent variables. Our results suggest that vegetable intake should be increased and saturated fat (dairy product) intake decreased to lower insulin resistance in young women.

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アポリポ蛋白A-I, A-II, A-IV

Apolipoprotein A-I, A-II, A-IV

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Key words：HDL, コレステロール逆転送, 冠動脈疾患, アポリポ蛋白A-I, A-II, A-IV

1. 概 説

HDL-コレステロール(HDL-C)は冠動脈疾患の独立した負の危険因子である[1]. HDL粒子のアポ蛋白の約70％はアポリポ蛋白(アポ)A-I, 20％はアポA-IIであり, 少量のアポA-IVも存在する(**表1**). アポA-Iは肝および小腸で, アポA-IIは肝臓で, アポA-IVは主に小腸で合成される.

アポA-IはHDLの構造蛋白であり, lecithin-cholesterol acyltransferase(LCAT)の活性化, 末梢組織からのコレステロールの引き抜き, HDL受容体のリガンドなど, コレステロールの逆転送系に重要な役割を果たしている.

アポA-IIの生理機能は, 不明な点が多い[2]が, *in vitro*では肝性トリグリセリドリパーゼ(HTGL)を活性化し, LCATを抑制する. HDL粒子にはアポA-Iとアポ A-IIの両者を有する粒子(LpA-I/A-II)と, アポA-Iのみを有する粒子(LpA-I)の2種類が存在する. コレステロール逆転送機能の主体はLpA-Iに存在するので, アポA-IIはこの2つの粒子間のアポA-I分布を決定することにより, 間接的にHDLの機能を調節していると考えられる.

脂肪摂取に伴うカイロミクロン産生により小腸細胞で合成, 分泌されるアポA-IVの生理的な役割も明らかではない[3]. *in vivo*の研究で, アポA-IVは, アポA-Iがもつ LCAT活性化能の約20-40％の能力をもつことが示されている. また, マウスアポA-IVを過剰発現させたマウスでは, アポA-IVが動脈硬化を抑制することが示されている.

表1 アポリポ蛋白A-I, A-II, A-IVの概要

アポリポ蛋白	主な機能	存在する主なリポ蛋白	主な生成部位
A-I	LCATの活性化, HDLの構造維持 HDL受容体に対するリガンド	HDL, カイロミクロン	肝臓, 小腸
A-II	不明	HDL	肝臓>>小腸
A-IV	LCATの活性化, コレステロール逆転送促進 小腸から視床下部への満腹シグナル(？)	HDL, カイロミクロン	小腸

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2. 検査の目的

アポA-IはHDL-Cとともに冠動脈疾患の負の危険因子であり，冠動脈疾患をはじめとする動脈硬化性疾患の予防・進展抑制の指標となる．アポA-IはHDL-Cと強い相関があり（正常健常者で相関係数0.8-0.9），アポA-Iの測定はHDLを含めたリポ蛋白代謝，特にコレステロールの逆転送系の理解に役立つ．

血清アポA-IIの90％以上はHDLと結合しているが，HDL-Cとの相関は，アポA-Iより弱い（正常健常者で相関係数0.4-0.5）．アポA-IIは単独で測定するのではなく，HDL-C，アポA-Iなどと合わせて測定することによって，HDL粒子の質的変化，機能的変化をより明らかにできる．

アポA-IVは一般臨床では測定できない．また，その臨床的な意義も確立されていない．

3. 試料の採取方法，保存条件

アポA-IとアポA-IIは食事摂取には大きく影響を受けないが，12時間以上の絶食後の空腹時に採血し，血清または血漿の形で保存する．測定値は血清および血漿でほとんど差がない．4℃保存では14日以内に測定しなければならないが，−20℃保存では28日以上経過した後でも著しい測定値の変動は認められない．

4. 測 定 法

血中濃度の測定には多くの免疫化学的測定法があるが，国際的にはアポA-IとアポBを除いてまだ標準化されていない．アポA-IとアポA-IIの測定は免疫比濁法（TIA）[4]が主に用いられている．アポA-IVの測定法[3]としては，定量的免疫電気泳動法（ロケット法），酵素免疫法（enzyme-linked immunosorbent assay: ELISA），ラジオイムノアッセイ（radioimmunoassay: RIA）がある．

5. 基準値（表2）

アポA-Iの基準値は男性で100-150 mg/dL，女性で120-180 mg/dLである．アポA-IIは男女とも20-40 mg/dLである．健常成人におけるアポA-Iの平均値[4]は男性より女性が高いが，アポA-IIの平均値[4]は男女差が認められない．空腹時ヒト血漿アポA-IV濃度は平均約15 mg/dLである[5,6]．

表2 アポリポ蛋白A-I，A-II，A-IVの健常成人における基準値

アポリポ蛋白	基準値	男性	女性
A-I（mg/dL）	男性：100-150 女性：120-180	128±24	141±23
A-II（mg/dL）	20-40	30.5±5.5	30.1±5.1
A-IV（mg/dL）		14.6±4.2（全体）	

平均±標準偏差．

6. 生理的変動（測定に影響を及ぼす因子）

HDLの主要な構成蛋白であるアポA-Iは，HDL-Cの変動と同様に変化する．すなわち，アポA-Iの血中濃度は思春期以後，女性が男性に比べ高値である（**表2**）[4]．また，高脂肪食でアポA-Iは上昇し，高糖質食で低下する．喫煙，身体活動の低下と体重の増加によりアポA-Iは低下し，禁煙，有酸素運動と体重減少により上昇する．季節変動，加齢による変動も明らかではない．

アポA-IIは，アポA-Iと異なり，性差は認めない．加齢による変動も認められない．慢性アルコール飲酒者（肝機能正常者）では，アポA-Iと比較してアポA-IIの上昇率が高い．また，フィブラート投与により血清アポA-IとアポA-II濃度は上昇する．

7. 異常値を示す疾患

アポA-IはHDL-Cと同様に冠動脈疾患の独立した危険因子である．また，血清アポA-I，A-IIとHDL-Cを測定することにより，多様なHDL粒子の変化を明らかにし，更にリポ蛋白異常の特徴を明らかにすることができる．アポA-IとA-IIは，HDLと正の相関および血清トリグリセリドと負の相関を示し，コレステロール逆転送系を反映して，冠動脈疾患で低下する．

家族性複合型高脂血症(FCH)[7]において，HDL-Cが低値にもかかわらず，血清アポA-IIの高値が報告された．FCHは，脂質代謝異常(高TG，低HDL，高アポB)のみならず，メタボリックシンドロームの特徴を数多く備えており，冠動脈疾患患者に多く認められる．FCHにおけるアポA-IIの有意の予知因子は血清TGであった[7]．一方，アポA-IIが冠動脈疾患の独立した負の危険因子であることが最近報告された[8]．アポA-IIのアテローム硬化に与える影響はいまだ確立されていない．

アポA-IとA-IIが高値を示す疾患には，原発性高HDL血症，CETP欠損症，HTGL欠損症，原発性胆汁性肝硬変の一部がある．アポA-IとA-IIの中等度低値は，高トリグリセリド血症，種々の肝疾患(急性肝炎，慢性肝炎，肝硬変，閉塞性黄疸)，コントロール不良の糖尿病，腎不全，薬物(プロブコール)でみられる．高度低値を示す疾患には，それぞれのアポリポ蛋白欠損症，LCAT欠損症，フィッシュ・アイ病，Tangier病がある．

アポA-IVがごく初期の腎機能障害で増加し[5]，慢性腎臓病の進行の独立した予知因子であることが報告された[6]．一方，大血管障害を合併する2型糖尿病患者[9]においてアポA-IVの高値が，冠動脈疾患において低値[10]が報告された．前者は2型糖尿病における腎機能障害を反映しているものと推察される．

血清アポA-IVは長鎖脂肪酸摂取，特に不飽和脂肪酸により，著明に増加する[11]．しかし，中鎖脂肪酸ではほとんど増加しない．また，血清アポA-IVは，脂肪吸収障害(無βリポ蛋白血症，低βリポ蛋白血症，慢性膵炎など)において著明に減少する．動物実験での成績ではあるが，アポA-IVは消化管由来の満腹因子の一つである[12]．重症肥満でみられる食欲の亢進が胃バイパス術後に消失し，このとき血清アポA-IVの著明な増加が体重の減少に伴って報告された[13]．

種々の疾患に伴う2次性の低下に対しては，原因疾患の治療をするが，高度のものはほとんどが遺伝性疾患に伴うもので，現在のところは診断にとどまる．

8. 関連検査項目

アポA-IとA-IIはHDLの構成蛋白であるので，HDL-C，LCAT活性，CETP活性とともにトリグリセリドを同時に測定する．また，中等度までの低値を示す場合には肝機能検査を，高度な高・低値を示す場合には関連する蛋白・酵素についての家系調査も併せて検査するべきである．

アポB/A-I比がリポ蛋白の脂質やLDL/HDL比より簡潔で，精度の高い新しい危険因子であることが判明した[14]．すなわち，この比が高ければ高いほど冠動脈疾患のリスクが高いといえる．

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Pleiotropic Effects of Mitiglinide in Type 2 Diabetes Mellitus

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This study investigated the effects of mitiglinide in 16 patients with type 2 diabetes mellitus treated with 30 mg/day mitiglinide, divided into three doses given just before each meal, for approximately 12 months. A 450 kcal meal tolerance test was performed at baseline and after 3, 6 and 12 months, and levels of plasma glucose and immunoreactive insulin were measured. Various parameters of glucose metabolism and lipid metabolism, urinary albumin and markers of atherosclerosis, coagulation and fibrinolysis were also determined. Mitiglinide showed a rapid stimulatory effect on insulin secretion and reduced the levels of plasma glucose. The free fatty acid level significantly decreased at 60 min after the meal tolerance test. Mitiglinide also significantly lowered glycosylated haemoglobin and raised 1,5-anhydroglucitol after 6 months, and significantly decreased urinary albumin after 12 months. These data indicate that mitiglinide may have beneficial effects not only on glycaemic control but also on lipid metabolism and urinary albumin excretion, and may have a role in the prevention of the vascular complications of diabetes.

KEY WORDS: TYPE 2 DIABETES MELLITUS; MITIGLINIDE; MEAL TOLERANCE TEST; GLYCAEMIC CONTROL; LIPID METABOLISM; URINARY ALBUMIN

Introduction

Patients with diabetes mellitus are at increased risk from cardiovascular-related morbidity and mortality compared with healthy individuals.[1] An association between the post-prandial state of metabolism and atherogenesis has been demonstrated[2,3] and has also been observed in patients with diabetes mellitus.[4,5] It has been suggested that post-prandial hyperglycaemia may be an independent risk factor for cardiovascular disease.[6] Moreover, large-scale clinical trials such as the Diabetes Epidemiology: Collaborative analysis Of Diagnostic criteria in Europe (DECODE) study have shown that post-prandial hyperglycaemia is a risk factor for arteriosclerosis, independent of other risk factors such as hypertension and hyperlipidaemia.[7,8] In the Study to Prevent Non-Insulin-Dependent Diabetes Mellitus (STOP-NIDDM), treatment with an α-glucosidase inhibitor in patients with

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impaired glucose tolerance not only reduced conversion to type 2 diabetes, but was also associated with a reduction in the risk of a cardiovascular event.[9] These results indicate the importance of treatment of post-prandial hyperglycaemia in the early stages of diabetes mellitus. Although sulphonylureas have been commonly used for the treatment of diabetes mellitus, they do not act rapidly enough to increase glucose-stimulated insulin secretion after a meal and are, therefore, insufficient to control post-prandial hyperglycaemia. Since it has been shown that sulphonylureas can easily cause prolonged hypoglycaemia and weight gain, they must be used carefully in the early stages of type 2 diabetes mellitus.[10,11]

Mitiglinide calcium hydrate, a derivative of benzylsuccinic acid, closes ATP-dependent K^+ channels by selectively binding to the sulphonylurea receptor on pancreatic β-cells, which leads to opening of the voltage-dependent calcium channels and induces insulin secretion.[12] The pharmacokinetic properties of mitiglinide include rapid absorption and elimination. Thus mitiglinide provokes a rapid and short-acting insulin secretion that improves post-prandial hyperglycaemia, mimicking normal physiological insulin secretion and glucose metabolism in healthy individuals. Due to its shorter duration of action, mitiglinide has a low risk of hypoglycaemia compared with other insulin secretagogues.[13] Moreover, it has been reported that the suppression of post-prandial hyperglycaemia with mitiglinide prevents post-prandial increases in oxidative stress and markers of inflammation in patients with diabetes mellitus.[14] In the present study in patients with type 2 diabetes mellitus, the effects of mitiglinide on fasting and post-prandial plasma glucose were investigated together with its pleiotropic actions on various parameters of lipid metabolism and urinary albumin excretion, and on markers of atherosclerosis, coagulation and fibrinolysis.

Patients and methods

PATIENTS

Patients with type 2 diabetes mellitus who were routinely and regularly attending the Diabetes Clinic of Hyogo College of Medicine, Hyogo, Japan, were recruited to the study between November 2004 and March 2006. The study protocol was approved by the Institutional Review Board of Hyogo College of Medicine and written informed consent was obtained from all the study participants.

MITIGLINIDE TREATMENT

Each patient received 30 mg/day mitiglinide, divided into three doses given just prior to each meal, for approximately 12 months. Patients continued on their usual treatment/diet and exercise during the course of the study. No additions or changes to other hypoglycaemic agents, antihypertensive agents, antiplatelet agents or antilipidaemic agents were made during the study period.

ASSESSMENTS

Meal tolerance tests using meals providing 450 kcal of energy, of which 50% was carbohydrate, 35% fat and 15% protein, were performed at baseline and after 3, 6 and 12 months. Patients were required to fast from 21:00 h on the day before each meal tolerance test. For each test, plasma levels of fasting glucose, and 30 and 60 min post-prandial plasma glucose levels, immunoreactive insulin (IRI) and free fatty acids (FFAs) were determined. In addition, the following variables were measured before and after mitiglinide treatment using

standard laboratory techniques: body weight; body mass index (BMI); waist girth; waist–hip ratio; body fat composition; blood pressure; glycosylated haemoglobin (Hb_{A1c}); 1,5-anhydroglucitol (1,5-AG); homeostasis model assessment insulin resistance index (HOMA-R) and homeostasis model assessment β-cell function (HOMA-β);[15] plasma levels of total cholesterol, triglyceride, high-density lipoprotein (HDL)-cholesterol, high-sensitivity C-reactive protein (hs-CRP), adiponectin, tumour necrosis factor-α (TNF-α), plasminogen-activator inhibitor type 1 (PAI-1), advanced glycation end-product (AGE; carboxymethyl lysine), receptor for AGE (RAGE), fibrinogen; and urinary albumin excretion. The levels of TNF-α, PAI-1, AGE and RAGE were measured using either a latex photometric immunoassay kit or enzyme-linked immunosorbent assay kit (SRL Inc., Tokyo, Japan), according to the manufacturer's instructions.

STATISTICAL ANALYSIS

Data are presented as the mean ± SE. The Wilcoxon signed-ranks test was used to compare differences from baseline in all study measurements at 3, 6 and 12 months post-treatment. Multiple regression analysis was performed to assess the combined influence of variables on changes in urinary albumin and 60-min post-prandial FFA levels in patients with type 2 diabetes mellitus treated with mitiglinide for 12 months. A *P*-value < 0.05 was considered to be statistically significant. All the statistical analyses were performed using StatView J-5.0 software (SAS Institute Inc., Cary, NC, USA).

Results

A total of 16 patients with type 2 diabetes mellitus were included in the study. Their characteristics and laboratory parameters at baseline are given in Table 1.

Treatment with mitiglinide for 12 months significantly reduced 30 and 60 min post-prandial plasma glucose levels from 203.0 ± 8.2 to 186.0 ± 8.4 mg/dl ($P < 0.05$) and from 241.9 ± 7.3 to 205.8 ± 10.6 mg/dl ($P < 0.005$), respectively (Fig. 1A). In addition, mitiglinide therapy significantly increased 30 and 60 min post-prandial IRI levels from 14.6 ± 2.0 to 31.1 ± 5.0 μU/ml ($P < 0.001$) and from 21.4 ± 3.2 to 28.2 ± 3.6 μU/ml ($P < 0.05$), respectively (Fig. 1B). Statistically significant improvements in Hb_{A1c} and 1,5-AG were observed after 3 and 6 months of treatment; however, these levels showed only a tendency for improvement after 12 months (Hb_{A1c} 7.5 ± 0.2% compared with 7.0 ± 0.2%; 1,5-AG 6.1 ± 1.1 μg/ml compared with 8.9 ± 1.6 μg/ml) (Fig. 2).

No significant changes were observed in body weight, BMI, waist girth, waist–hip ratio, body fat composition or systolic and

TABLE 1:
Baseline characteristics and laboratory parameters of the patients with type 2 diabetes mellitus who were included in the study ($n = 16$)

Age (years)	64.8 ± 2.0
Sex	
Male	12
Female	4
BMI (kg/m^2)	23.3 ± 1.0
Duration of diabetes (years)	9.7 ± 2.4
Hb_{A1c} (%)	7.5 ± 0.2
FPG (mg/dl)	155.3 ± 8.3
PPG (1 h)	241.9 ± 7.3
IRI (μU/ml)	6.8 ± 1.2
FFA (mEq/l)	0.78 ± 0.06
Urinary albumin (mg/g creatinine)	27.7 ± 7.2

Values are expressed as the mean ± SE or number of patients.
BMI, body mass index; Hb_{A1c}, glycosylated haemoglobin; FPG, fasting plasma glucose; PPG, post-prandial glucose; IRI, immunoreactive insulin; FFA, free fatty acid.

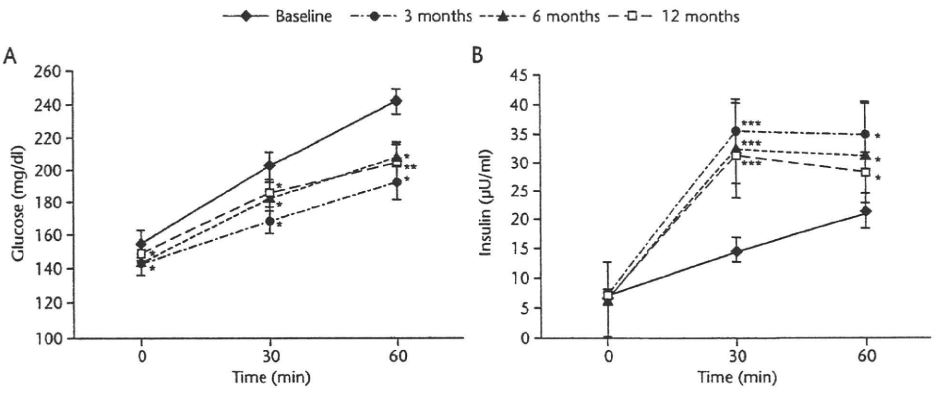

FIGURE 1: (A) Post-prandial plasma glucose levels and (B) post-prandial immunoreactive insulin levels in patients with type 2 diabetes mellitus treated with mitiglinide for 12 months (n = 16; *P < 0.05, **P < 0.005 and ***P < 0.001 versus baseline; mean ± SE)

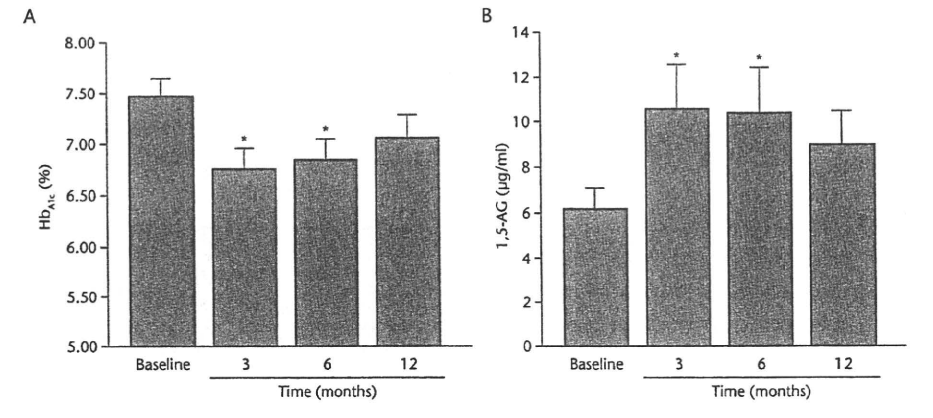

FIGURE 2: Levels of (A) glycosylated haemoglobin (Hb_{A1c}) and (B) 1,5-anhydroglucitol (1,5-AG) in patients with type 2 diabetes mellitus treated with mitiglinide for 12 months (n = 16; *P < 0.05 versus baseline; mean ± SE)

diastolic blood pressure after 12 months of treatment with mitiglinide (Table 2).

No significant changes were observed in fasting serum lipid levels (total cholesterol, triglyceride, HDL-cholesterol and FFAs) (Table 3; Fig. 3A), whereas the 60 min post-prandial FFA level was significantly reduced from 0.52 ± 0.06 to 0.30 ± 0.03 mEq/l (P < 0.01) after 12 months of treatment with mitiglinide (Fig. 3B). Urinary albumin excretion was also significantly reduced from 27.7 ± 7.2 at baseline to 20.7 ± 5.4 mg/g creatinine at 12 months (P < 0.05) (Fig. 4). After 12 months of treatment, HOMA-R was reduced from 2.6 ± 0.5 to 2.3 ± 0.4, and HOMA-β was increased from 29.7 ± 6.2 to 33.4 ± 11.5; however, these changes were not statistically significant (Table 4). Treatment

TABLE 2:
Body weight and blood pressure parameters of the patients with type 2 diabetes mellitus treated with mitiglinide for 12 months (n = 16)

Parameter	Baseline	After mitiglinide treatment
Body weight (kg)	60.7 ± 2.4	61.6 ± 2.1
Body mass index (kg/m^2)	23.3 ± 1.0	23.7 ± 1.0
Waist girth (cm)	82.9 ± 2.4	82.8 ± 2.0
Waist–hip ratio	0.92 ± 0.01	0.93 ± 0.01
Body fat composition (%)	24.1 ± 2.4	24.6 ± 1.8
Systolic blood pressure (mmHg)	125.6 ± 4.9	122.4 ± 4.3
Diastolic blood pressure (mmHg)	72.4 ± 3.2	70.2 ± 3.2

Values are expressed as the mean ± SE.
No statistically significant between-group differences were found for any of the parameters ($P > 0.05$; Wilcoxon signed-ranks test).

TABLE 3:
Fasting serum lipid levels in the patients with type 2 diabetes mellitus treated with mitiglinide for 12 months (n = 16)

Parameter	Baseline	After mitiglinide treatment
Total cholesterol (mg/dl)	219.1 ± 8.3	222.9 ± 10.6
Triglycerides (mg/dl)	117.4 ± 13.7	119.1 ± 12.5
HDL-cholesterol (mg/dl)	58.3 ± 3.6	58.6 ± 3.8
Free fatty acids (mEq/l)	0.78 ± 0.06	0.72 ± 0.07

Values are expressed as the mean ± SE.
No statistically significant between-group differences were found for any of the parameters ($P > 0.05$; Wilcoxon signed-ranks test).
HDL, high density lipoprotein.

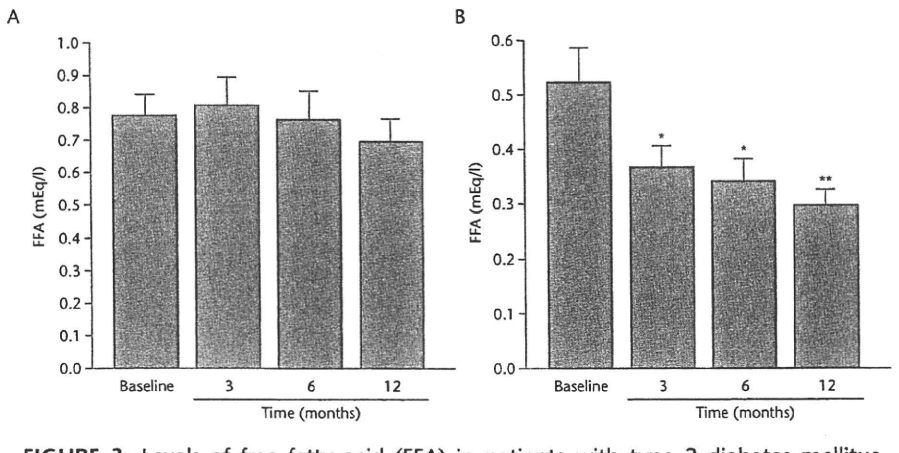

FIGURE 3: Levels of free fatty acid (FFA) in patients with type 2 diabetes mellitus treated with mitiglinide for 12 months (A) after fasting and (B) 60 min after meal tolerance test (n = 16; *$P < 0.05$ and **$P < 0.01$ versus baseline; mean ± SE)

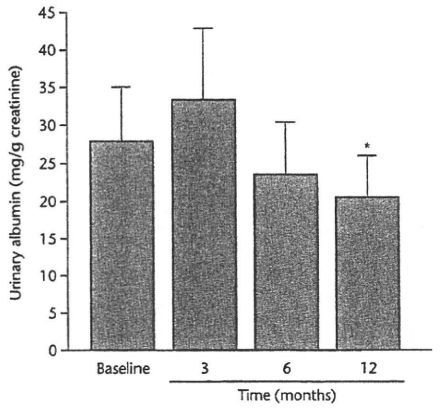

FIGURE 4: Urinary albumin excretion in patients with type 2 diabetes mellitus treated with mitiglinide for 12 months ($n = 16$; *$P < 0.05$ versus baseline; mean ± SE)

with mitiglinide for 12 months decreased levels of hs-CRP, TNF-α, PAI-1, RAGE and fibrinogen, and increased levels of adiponectin and AGE; however, none of these changes was statistically significant (Table 4).

Multiple regression analysis was used to determine the effect of variables such as HOMA-R, PAI-1 and AGE at baseline on changes in the 60 min post-prandial FFA level and urinary albumin excretion after 12 months of treatment with mitiglinide. The results showed that the 60-min post-prandial FFA level was reduced most commonly in patients with a lower PAI-1 or AGE level at baseline (Table 5) and that urinary albumin excretion was improved most commonly in patients with a higher HOMA-R and lower PAI-1 levels at baseline (Table 6). No serious hypoglycaemia was observed during the study period.

Discussion

In the present study, mitiglinide therapy resulted in a statistically significant reduction in post-prandial hyperglycaemia by significantly increasing glucose-stimulated insulin secretion after a meal without causing serious hypoglycaemia. It has been previously reported that mitiglinide reduces post-prandial hyperglycaemia by improving early insulin secretion in type 2 diabetes patients, mimicking normal

TABLE 4:
Markers of atherosclerosis, coagulation and fibrinolysis in the patients with type 2 diabetes mellitus treated with mitiglinide for 12 months ($n = 16$)

Marker	Baseline	After mitiglinide treatment
HOMA-R	2.6 ± 0.5	2.3 ± 0.4
HOMA-β	29.7 ± 6.2	33.4 ± 11.5
hs-CRP (ng/ml)	1316.5 ± 581.9	719.6 ± 108.8
Adiponectin (μg/ml)	8.6 ± 1.0	8.8 ± 1.1
TNF-α (pg/ml)	2.2 ± 0.7	1.8 ± 0.2
PAI-1 (ng/ml)	40.7 ± 5.7	39.9 ± 3.5
AGE (mU/ml)	3.2 ± 0.2	3.4 ± 0.3
RAGE (pg/ml)	1124.9 ± 113.0	1025.6 ± 101.2
Fibrinogen (mg/ml)	285.5 ± 19.5	281.7 ± 13.8

Values are expressed as the mean ± SE.
No statistically significant between-group differences were found for any of the parameters ($P > 0.05$; Wilcoxon signed-ranks test).
HOMA-R, homeostasis model assessment insulin resistance index; HOMA-β, homeostasis model assessment β-cell function; hs-CRP, high-sensitivity C-reactive protein; TNF-α, tumour necrosis factor-α; PAI-1, plasminogen activator inhibitor; AGE, advanced glycation end-products; RAGE, receptor for AGE.

TABLE 5:
Multiple regression analysis of changes in levels of 60-min post-prandial free fatty acids in the patients with type 2 diabetes mellitus treated with mitiglinide for 12 months (n = 16)

Independent variable	β	SE	Standardized β	Statistical significance[a]
PAI-1	0.006	0.002	0.541	P = 0.0224
AGE	0.123	0.053	0.486	P = 0.0369

[a]Multiple regression analysis was performed in order to assess the combined influence of variables on changes in levels of 60-min post-prandial free fatty acids in patients with type 2 diabetes mellitus treated with mitiglinide for 12 months. For independent variables, the baseline values were used; for dependent variables, the change in 60-min post-prandial free fatty acids after 12 months' mitiglinide therapy was used.
PAI-1, plasminogen-activator inhibitor type 1; AGE, advanced glycation end-products.

TABLE 6:
Multiple regression analysis of changes in levels of urinary albumin in the patients with type 2 diabetes mellitus treated with mitiglinide for 12 months (n = 16)

Independent variable	β	SE	Standardized β	Statistical significance[a]
HOMA-R	−5.681	1.195	−0.737	P = 0.0005
PAI-1	0.250	0.094	0.407	P = 0.0212

[a]Multiple regression analysis was performed to assess the combined influence of variables on changes in levels of urinary albumin in patients with type 2 diabetes mellitus treated with mitiglinide for 12 months. For independent variables, the baseline values were used; for dependent variables, the change in urinary albumin after 12 months' mitiglinide therapy was used.
HOMA-R, homeostasis model assessment insulin resistance index; PAI-1, plasminogen-activator inhibitor type 1.

physiological insulin secretion.[16] In addition, due to its shorter duration of action, mitiglinide is less likely to cause hypoglycaemia.[13] In the present study, Hb_{A1c} and 1,5-AG levels were significantly improved up to 6 months after initiation of treatment; however, these statistically significant improvements were not maintained at 12 months of treatment.

Sulphonylurea agents, which stimulate basal insulin secretion for long periods, often cause weight gain.[10,11] In contrast, because it stimulates rapid and short-lasting insulin secretion, 12 months of treatment with mitiglinide induced little change in body weight, BMI, waist girth, waist–hip ratio and body fat composition.

Previously, the effect of a glinide on post-prandial hyperlipidaemia, as well as hyperglycaemia, was investigated in Otsuka Long-Evans Tokushima Fatty (OLETF) rats, a model of spontaneous type 2 diabetes in which early insulin secretion is impaired. The results indicated that an increase in early post-prandial insulin secretion induced by glinides improved not only post-prandial hyperglycaemia but also post-prandial hyperlipidaemia.[17,18] The clinical effects of nateglinide, which also produces rapid insulin secretion, on post-prandial hyperlipidaemia have been reported.[19] No statistically significant changes in fasting total cholesterol, triglyceride, HDL-cholesterol or FFAs were observed after 12 months of treatment with mitiglinide in the present study, whereas there was a statistically significant decrease in 60-min post-prandial FFAs, suggesting that this drug may reduce lipotoxicity. FFAs may cause or aggravate type 2 diabetes mellitus and metabolic syndrome via inhibition of insulin action in insulin-sensitive organs such as

adipose tissue. The phenomenon, so-called lipotoxicity, is one of the pathogenic mechanisms of insulin resistance associated with visceral obesity.[20] The 60-min post-prandial FFA level was reduced most commonly in patients with lower PAI-1 or AGE levels at baseline, indicating that mitiglinide may, therefore, be more effective in the early stages of diabetes with less advanced complications.

Mitiglinide therapy for 12 months significantly reduced urinary albumin excretion in the present study. It has previously been reported that mitiglinide prevents the increase in oxidative stress associated with post-prandial hyperglycaemia.[14] Thus, mitiglinide may reduce urinary albumin excretion by exerting an antioxidant effect. Furthermore, it has been reported that FFA-induced oxidative stress contributes to vascular endothelial dysfunction,[20] which suggests the possibility that mitiglinide reduces this FFA-induced vascular endothelial dysfunction, resulting in a reduction in urinary albumin excretion. Urinary albumin excretion was improved in patients with a higher HOMA-R level and lower PAI-1 level after 12 months of treatment in the present study. Thus, mitiglinide may have a tendency to reduce urinary albumin excretion in patients with increased insulin resistance and in those with impaired coagulation and fibrinolysis.

Treatment with mitiglinide did not significantly improve the levels of hs-CRP, adiponectin, TNF-α, PAI-1, AGE, RAGE or fibrinogen in the present study. Significant changes may not have been seen because these values were within normal ranges at baseline. Thus, further investigation in patients with aggravated arteriosclerosis is needed to clarify the anti-arteriosclerosis effects of mitiglinide.

In conclusion, mitiglinide significantly increased post-prandial insulin secretion and decreased post-prandial plasma glucose and FFAs, without causing weight gain or severe hypoglycaemia, suggesting that this agent may be indicated in the initial treatment of type 2 diabetes mellitus. Furthermore, mitiglinide showed promising pleiotropic effects, including an improvement in lipid metabolism, and may have a role in the prevention of vascular complications such as diabetic nephropathy in patients with type 2 diabetes.

Conflicts of interest

The authors had no conflicts of interest to declare in relation to this article.

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Effects of gliclazide on platelet aggregation and the plasminogen activator inhibitor type 1 level in patients with type 2 diabetes mellitus

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Abstract

Vascular complications are a common factor determining morbidity and mortality of diabetic patients. In vitro studies have revealed that gliclazide has antiplatelet activities. To clinically assess this action, we measured the effects of gliclazide on platelet activities and abnormal fibrinolysis in patients with type 2 diabetes mellitus. We studied 14 patients aged 38 to 72 years (9 men and 5 women) with type 2 diabetes mellitus who have been treated with glibenclamide in our hospital for more than 6 months. We switched from glibenclamide to gliclazide using the average ratio of the respective doses, 2.5 vs 40 mg. We titrated the dose of gliclazide to keep the glycemic control at the same level as the previous (glibenclamide) treatment. We measured 10 μmol/L serotonin–induced or 0.5 μmol/L adenosine diphosphate (ADP)–induced platelet aggregate formation by particle counting using light scattering at baseline and up to 6 months after the switch. After switching to gliclazide, platelet aggregate formation induced by serotonin was significantly reduced ($P < .05$, compared with the levels observed after glibenclamide treatment). The body mass index, fasting plasma glucose, immunoreactive insulin, homeostasis model assessment of insulin resistance, hemoglobin A_{1c} (HbA_{1c}), total cholesterol, triglycerides, high-density lipoprotein cholesterol, prothrombin time, activated partial thromboplastin time, fibrinogen, thrombin-antithrombin III complex, plasmin-α2-plasmin inhibitor complex, and plasma plasminogen activator inhibitor type 1 (PAI-1) were not changed. In the group with improved HbA_{1c} (n = 5), ADP-induced platelet aggregate formation and plasma PAI-1 level were significantly reduced ($P < .05$, compared with the group with aggravated HbA_{1c}, n = 9). Multiple regression analysis showed that percentage change of ADP-induced platelet aggregate formation (standardized $\beta = 0.540$, $P < .05$) was independently associated with percentage change of plasma PAI-1 level in addition to percentage change of HbA_{1c} (standardized $\beta = 0.657$, $P < .05$) ($R = 0.939$, $P < .05$) after switching to gliclazide. The other independent variants, like the final dose of gliclazide, homeostasis model assessment of insulin resistance, percentage change of prothrombin time, activated partial thromboplastin time, and total cholesterol, were not significantly associated with the percentage change of plasma PAI-1 level. These results indicate that gliclazide inhibits platelet aggregation via the serotonin pathway, independently of the metabolic control per se. Furthermore, in the patients with improved glycemic control, gliclazide could inhibit ADP-induced platelet aggregation and reduce PAI-I level. Taken together, the results show that gliclazide may be more useful for the prevention of diabetic vascular complications than glibenclamide.

1. Introduction

Atherosclerotic complications play a crucial role in the prognosis of type 2 diabetes mellitus (DM). It is fully recognized that long-term macrovascular complications are common factors determining morbidity and mortality in the diabetic population. The Diabetes Control and Complications Trial and UK Prospective Diabetes Study indicate a consistent relationship between hyperglycemia and the incidence of chronic vascular complications in type 1 and type 2 DM, respectively [1,2]. Platelet function in DM patients is enhanced and is correlated with both agonist-induced and spontaneous aggregation [3]. It is thought that long-term exposure to high glucose levels may enhance

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Table 1
Characteristics of enrolled patients

Age (y)	61.5 ± 2.6
Sex (M/F)	9/5
Height (cm)	165.3 ± 2.9
Weight (kg)	62.0 ± 3.1
BMI (kg/m^2)	22.5 ± 0.8
Duration of DM (y)	12.0 ± 1.8
HbA_{1c} (%)	7.4 ± 0.2
FPG (mg/dL)	165.0 ± 7.0
IRI (μU/mL)	7.8 ± 1.6
HOMA-R	3.0 ± 0.6

Data are expressed as mean ± SEM.

platelet function in DM patients. Moreover, rapid alterations of platelet aggregability in acute hyperglycemia have also been reported [4]. Intraplatelet serotonin (5-hydroxytryptamine; 5-HT) content is diminished and plasma levels of 5-HT are increased in DM patients [5].

This increase in plasma 5-HT may reflect enhanced release of platelet 5-HT by hyperactive platelets that may contribute to the pathogenesis of atherosclerosis. The measurement of 5-HT–induced platelet aggregation is therefore a useful method to evaluate the risk of diabetic complications in DM patients [5].

A technique for studying platelet aggregation by particle counting using light scattering may detect subtle changes in platelet activation [6]. Hypercoagulability and decreased fibrinolysis, including increased plasma plasminogen activator inhibitor type 1 (PAI-1) level, are often found and are considered to be risk factors of cardiovascular diseases and glucose intolerance, especially in patients with non–insulin-dependent DM [7]. Gliclazide is a second-generation sulfonylurea with the potency of free radical scavenger activity. Some studies have shown that gliclazide has beneficial effects on the hemorrheologic abnormalities seen in diabetic vascular disease [8-12].

To assess this clinically, we measured platelet activities and fibrinolysis in patients with type 2 DM treated with gliclazide; and we compared the results with those obtained in patients treated with glibenclamide.

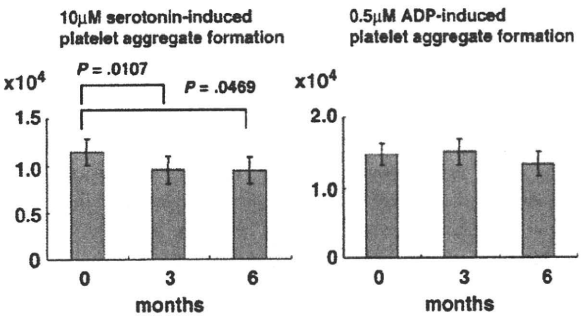

Fig. 1. Effects of gliclazide on platelet aggregation. Data are expressed as mean ± SEM.

2. Subjects and methods

2.1. Subjects

Fourteen patients with type 2 DM (9 men and 5 women; age [mean ± SEM], 61.5 ± 2.6) were randomly chosen as subjects. They were admitted to our metabolic ward between the years 2001 and 2002. Diagnosis of diabetes was based on World Health Organization 1998 criteria. All patients were treated with diet and glibenclamide. All the procedures in the study and the protection of the patients' private information were approved by the ethical committee of Hyogo College of Medicine. Informed consent was obtained from each patient before enrollment in the study.

2.2. Experimental protocol

We switched from glibenclamide to gliclazide using the average ratio of the respective doses, 2.5 vs 40 mg (1.25-20 mg in 2 patients, 2.5-40 mg in 6 patients, 5.0-80 mg in 2 patients, and 7.5-120 mg in 3 patients). We titrated the dose of gliclazide to keep the glycemic control at the same level as in the glibenclamide control. Patients' blood was assayed 3 times: before switching from glibenclamide to gliclazide and then 3 and 6 months after switching.

2.3. Blood sample preparation

Blood was collected in fasting condition on the respective mornings. Venous blood was drawn into 3.8% sodium citrate (1:9 vol/vol). Platelet-rich plasma (PRP) and platelet-poor plasma were obtained by centrifugation of the citrated blood at room temperature for 10 minutes at 150g and for 15 minutes at 3000g, respectively. The platelet count in PRP was adjusted to 2×10^{11}/L with platelet-poor plasma.

For the measurement of PAI-1, blood was centrifuged for 15 minutes at 3000g; and the supernatant was kept at −80 °C until assayed. Fasting plasma glucose (FPG), immunoreactive insulin (IRI), hemoglobin A_{1c} (HbA_{1c}), fasting serum concentrations of total cholesterol (T-Chol), triglycerides (TG), high-density lipoprotein cholesterol (HDL-Chol), prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen (Fbg), thrombin-antithrombin III complex (TAT), and plasmin-α2-plasmin inhibitor complex (PIC) were also measured. Total cholesterol, TG, and HDL-

Table 2
Effects of gliclazide on metabolic factors

	Before	3 mo	6 mo
BMI (kg/m^2)	22.5 ± 0.8	22.3 ± 0.8	21.9 ± 0.8
HbA_{1c} (%)	7.4 ± 0.2	8.0 ± 0.3	7.7 ± 0.3
FPG (mg/dL)	165.0 ± 7.0	166.0 ± 9.9	170.0 ± 8.0
IRI (μU/mL)	7.8 ± 1.6	7.5 ± 1.5	7.4 ± 1.1
HOMA-R	3.0 ± 0.6	3.0 ± 0.8	3.0 ± 0.4
T-Chol (mg/dL)	205.0 ± 8.3	205.0 ± 8.7	201.0 ± 9.4
TG (mg/dL)	121.0 ± 22.1	102.0 ± 10.6	135.0 ± 19.3
HDL-Chol (mg/dL)	50.0 ± 2.6	50.0 ± 2.3	48.0 ± 1.8

Table 3
Effects of gliclazide on coagulation test and PAI-1

	Before	3 mo	6 mo
PT-INR	0.93 ± 0.01	0.92 ± 0.01	0.92 ± 0.02
APTT (s)	25.8 ± 0.7	25.8 ± 0.6	26.6 ± 0.4
Fbg (mg/dL)	300.0 ± 20.7	301.0 ± 9.5	340.0 ± 11.8
TAT (ng/mL)	50.2 ± 22.2	15.3 ± 5.4	25.8 ± 7.8
PIC (μg/mL)	0.8 ± 0.1	0.8 ± 0.1	1.3 ± 0.3
PAI-1 (ng/mL)	42.0 ± 5.6	35.4 ± 4.9	36.4 ± 5.3

Data are expressed as mean ± SEM. INR indicates international normalized ratio.

Chol were assayed using an autoanalyzer (JCA-BM 2250; Nihon Denshi, Akishima, Tokyo, Japan), while HbA_{1c} was measured by high-performance liquid chromatography (HLC-723G7 system; Tosoh, Tokyo, Japan). The subjects were then divided into 2 groups depending on whether their HbA_{1c} levels were improved or aggravated 6 months after switching from glibenclamide to gliclazide.

2.4. Platelet aggregation

Platelet aggregation was monitored with an AG10 aggregometer (Kowa, Tokyo, Japan) that determines the size and number of platelet aggregates based on particle counting using light scattering [6,13]. A laser beam (675 nm) is passed through a platelet suspension, and the intensity of light scattering provides information on the number and size of aggregates. Data were recorded as a 2-dimensional graph showing the change over time of total light intensity expressed as cumulative summation. The total light intensities of small aggregates were determined. Particles with an intensity of 25 to 400 mV represent small aggregates consisting of less than 100 platelets. Platelet-rich plasma (180 μL) was placed in a cuvette and incubated for 3 minutes at 37°C while rotating at 1000 rpm. Subsequently, 20 μL of 5-HT (final concentration, 10 μmol/L) or adenosine diphosphate (ADP) (0.5 μmol/L) was added; and the formation of platelet aggregates was monitored for 5 minutes. For this experiment, we determined the peak level of aggregate formation.

2.5. Statistical analysis

Values are presented as means ± SEM. Correlations were assessed using Spearman rank correlation test. Multiple regression analysis was performed to assess the combined influence of variables on percentage change of plasma PAI-1 levels. The Wilcoxon signed rank test or the Mann-Whitney U test were used for comparison. Differences were considered significant at $P < .05$. All the statistical analyses were performed using StatView J-5.0 software (SAS Institute, Berkeley, CA).

3. Results

3.1. Clinical characteristics of the patients

The clinical characteristics of the enrolled patients in the study are summarized in Table 1. The mean FPG was 165.0 ± 7.0 mg/dL (reference range, 70-110 mg/dL), and HbA_{1c} was 7.4% ± 0.2% (reference range, 4.0%-5.4%).

3.2. Change of platelet aggregation and metabolic factors after switching to gliclazide

After switching from glibenclamide to gliclazide, platelet aggregate formation induced by serotonin was significantly reduced (P = .0107, compared with glibenclamide treatment) after 3 months and (P = .0469, compared with glibenclamide treatment) after 6 months, although the ADP-induced platelet aggregate formation was not changed at all (Fig. 1). The switch from glibenclamide to gliclazide did not modify body mass index (BMI), FPG, IRI, homeostasis model assessment of insulin resistance (HOMA-R), HbA_{1c}, T-Chol, TG, and HDL-Chol (Table 2).

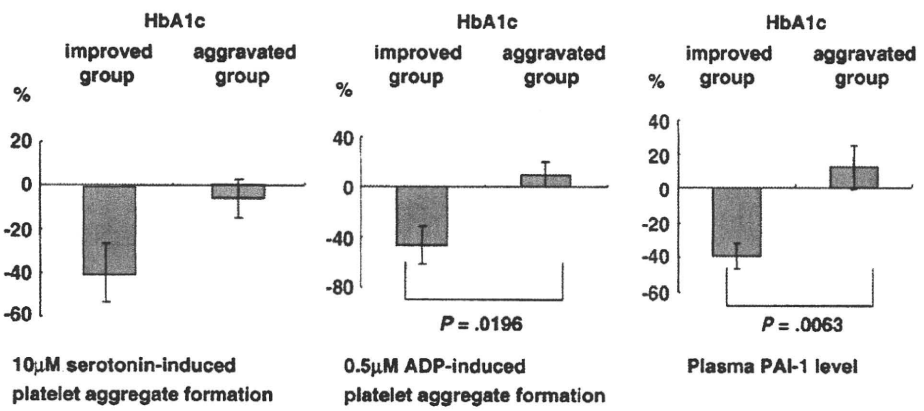

Fig. 2. Percentage change of platelet aggregate formations by 10 μmol/L serotonin or 0.5 μmol/L ADP and plasma PAI-1 level depend on the change of glycemic control after switching to gliclazide. Data are expressed as mean ± SEM.