Ⅳ．治　療
④糖尿病新規発症に及ぼす降圧薬の影響

New onset of diabetes and antihypertensive drugs

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■　はじめに

糖尿病は強力な心血管リスクであり，糖尿病が存在するだけで心筋梗塞の既往をもつ患者のリスクと同等となることが知られている[1]。以前より高血圧患者は，インスリン抵抗性を伴うことが報告されているが[2]，実際代謝症候群を有する患者も多く，糖尿病発生のリスクが高い。したがって糖代謝を悪化させるような治療介入は，もしそれが降圧によるリスク軽減に影響するなら避けるべきであるが，その一方で高血圧患者の予後に関しては，基本的に使用する降圧薬の種類を問わず降圧そのものに依存する部分が多い。これは最近のさまざまな臨床試験の結果が示唆している。この総説では，①高血圧治療中の糖尿病新規発症は心血管リスクを高めるか，②降圧利尿薬とβ遮断薬は糖尿病発生のリスクを高めるのか，③降圧薬の糖代謝への影響が降圧作用を超えて心血管リスクに影響するかの3点を議論したい。

■Ⅰ　高血圧治療中の糖尿病発生は心血管リスクとなるのか

最近，Verdecchiaらは795名の未治療高血圧患者を登録し，治療開始後のフォローアップ（中央値で3年後）で新たに糖尿病と診断された患者は，糖尿病と診断されなかった患者に比べ登録時糖尿病と診断された患者と同程度に心血管リスクが高いこと（図1），また糖尿病発症には治療前の血糖値と利尿薬の使用がリスクになると報告している[3]。引用した図は一見説得力に富む。しかし心血管イベントの判定が厳密ではなく，イベント発生数そのものが非常に少ない研究であり（脳卒中18例，心筋梗塞13例），しかも診断の妥当性を判定しにくい新たな狭心症の発生（15例）やTIA（10例）でイベント全体の1/3を占めるような結果であり，このような結論を引き出せるかどうか疑問である。確かに糖尿病は疑いもなく強力な心血管リスクであり，高血圧治療中に新たに発生した糖尿病とはいえ診断される以前からのインスリン抵抗性や血管内皮機能低下により動脈硬化は進行しているため，心血管リスクは高いであろう。したがって彼らの仮説は妥当なものであるが，残念ながらこの研究はそれを支持する結果を十分提供しているとは言いがたい。

しかしこの研究の問題点はむしろもう1つの結論，すなわち降圧利尿薬使用が糖尿病発症のリスクになり，ひいては心血管リスクになるという論調である。これは43名の新たな糖尿病を発症した患者と約700名の糖尿病を発症しなかった患者の降圧薬の頻度を比較し，利尿薬の使用が新たに発症した患者では多いという結果から引き出されたものである。しかし実際は，利尿薬のみならずカル

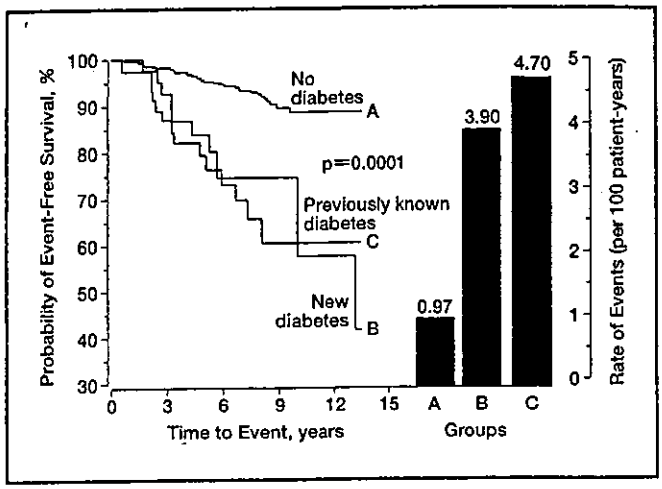

図1 Cardiovascular events in treated hypertensive subjects without diabetes (group A), new-onset diabetes (group B), and previously known diabetes (group C)

シウム拮抗薬やACE阻害薬も新規糖尿病発症患者で多く(図2)，要するに新規に糖尿病を発症するような患者では血圧のコントロールに多くの薬剤を要する，あるいは降圧利尿薬が必要とされるとも解釈できる。より多くの症例で検討すれば，ACE阻害薬が新規糖尿病の発生と関連があるという結果が出たかもしれない。したがってこの結論の根拠は脆弱であり，結果のサマリーだけが独り歩きして降圧における利尿薬の使用を理由なくためらわせることになる。

この問題にチャレンジした研究がDunderらによりもう1つ報告されている[4]。彼らは治療されている高血圧(主として利尿薬とβ遮断薬，n=291)と正常血圧者群(n=1,358)において治療中(10年間，正常者はもちろん治療していない)の血糖値の上昇の心筋梗塞発症への影響を検討している。高血圧治療群では経過観察中に血糖値が上昇し，それが心筋梗塞のリスクを37%増すことが報告された。この研究には大きな問題があり，なぜ質の高い臨床研究を掲載するBMJがこのような論文を受理したか理解に苦しむ。対照群との症例数の差が大きいのも問題であるが，最大の問題は対照を正常血圧者としているため，高血圧そのものの血糖値への影響を検討できていないことである。この論文の論調では，降圧利尿薬やβ遮断薬による血糖上昇は心筋梗塞のリスクになるので他剤を使用するべきであるというところに行き着く。実際この論文が出た日，多くの熱心なジュニアドクターが降圧利尿薬を処方から外そうとしたことが指導医により報告されている[5]。この行為は全く「エビデンスに基づいたもの」ではなく，ここに降圧薬の糖代謝への影響を過大評価する危険性——角を矯めて牛を殺すという言葉があてはまる——が集約されている。

そもそも2型糖尿病では診断される前にインスリン抵抗性や内皮機能低下などにより動脈硬化が進行していることが指摘されており，実際，前糖尿病状態とも考えられる代謝症候群の新血管リスクは高い。したがって発症した(むしろ新たに診断されたというべきか)糖尿病は心血管リスクをある程度増すことは明らかであるが，それがすべて使用する降圧薬に関連するという論調は誤りである。

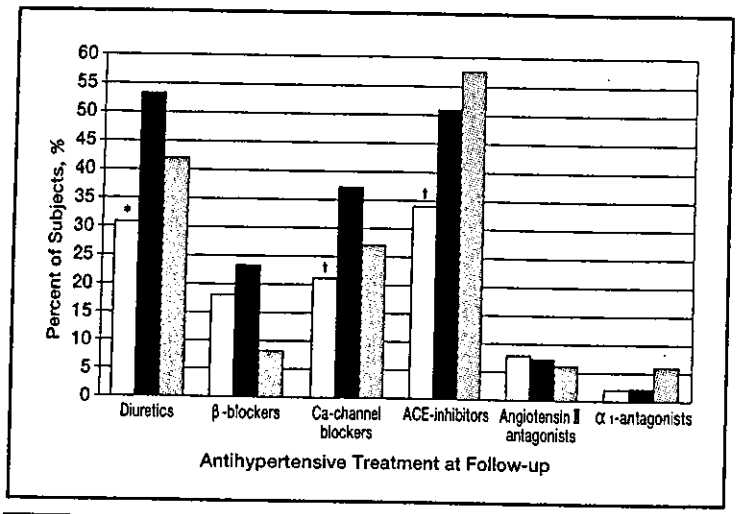

図2 Distribution of antihypertensive treatments at the follow-up visit in nondiabetic subjects, subjects with new-onset diabetes, and subjects with previously known diabetes
□：No diabetes, ■：New diabetes, ▨：Previously known diabetes
*$p<0.01$ vs. new diabetes, †$p<0.05$ vs. new diabetes

■Ⅱ 降圧利尿薬やβ遮断薬は糖尿病発生のリスクとなるのか

高用量の利尿降圧薬では，糖尿病の発生をはじめ種々の代謝面の副作用が報告されている。しかし低用量では必ずしもそうではない。SHEP研究によると，老齢者の収縮期性高血圧において，糖尿病は3年間の観察期間中に利尿薬群で1,631名中140例（8.6%），プラセボ群でも1,578名中118名（7.5%）に発症し有意差はなかった[6]。11,855名の血糖降下療法を開始した患者においても降圧薬服用（したがって高血圧そのもの）は有意な危険因子であったが，降圧薬間でのリスクの差は認めなかった[7]。12,550名の高血圧患者における前向き研究では，高血圧自体が新たな糖尿病発症のリスクになるが，利尿降圧薬そのものはリスクとならないと報告された[8]。この研究ではβ遮断薬の使用が糖尿病発症のリスクとなるとされているが，著者らは賢明にも，これを理由にβ遮断薬投与によってもたらされる多くの心疾患におけるbenefitを忘れてはならないと注意を喚起している。

CAPPP研究[9]ではカプトプリルとβ遮断薬，利尿薬などいわゆるconventional therapyを比較したが，糖尿病の発生は前者でやや少なかった（relative riskのCIは境界域[0.74-0.99]）。この研究では糖尿病発生が二次エンドポイントとして試験前に設定されている。INSIGHT研究ではカルシウム拮抗薬と降圧利尿薬を比較し，糖尿病の発生は降圧利尿薬群で多かったが，この研究のプロトコルは基本的に降圧利尿薬とカルシウム拮抗薬の単独治療を比較したもので，ハイドロクロロチアジドは25mgで開始され，効果不十分であれば増量し，その後基本的にβ遮断薬を併用している[10]。ハイドロクロロチアジドは25mg以上から代謝系副作用は増えるが，降圧効果は12.5mgと変わらないとされるため[11]，この使用法は現在では適切ではないと考えられる。したがって，より少量の降圧利尿薬を用いた併用療法では，このような糖尿病発生の差は生じない可能性がある。

ALLHAT研究では降圧利尿薬群で糖尿病の発生

表 降圧利尿薬臨床試験における新規糖尿病の発症

研究名	観察期間（年）	糖尿病発症（%） 利尿薬またはβ遮断薬	プラセボまたはACEI，CA，ARB	
SHEP	3	140/1,631 (8.6)	118/1,578 (7.5)	(diuretics vs placebo)
CAPPP	5	380/5,493 (6.9)	337/5,492 (6.1)	("conventional" vs ACEI)
NORDIL	5	251/5,471 (4.6)	216/5,410 (4.0)	("conventional" vs CA)
STOP-2	6	252/2,213 (11.4)	467/4,404 (10.6)	("conventional" vs CA/ACEI)
INSIGHT	4	137/3,164 (4.3)	96/3,147 (3.0)	(diuretics vs CA)
NICS-EH	5	4/210 (0.2)	0/204 (0)	(diuretics vs CA)
ALLHAT	4	302/2,606 (11.6)	154/1,567 (9.8)	(diuretics vs CA)
			119/1,464 (8.1)	(diuretics vs ACEI)
LIFE	5	319/3,959	241/4,019	(βblocker+diuretics vs ARB+diuretics)

ACEI：ACE阻害薬，CE：カルシウム拮抗薬，ARB：アンジオテンシンタイプ1受容体拮抗薬

が多いが，平均BMI 30kg/m²という肥満高血圧患者を対象にした試験であることや併用薬としてカルシウム拮抗薬，ACE阻害薬，ARBは使用できなかったことも考慮すべきである[12]。LIFE研究ではβ遮断薬＋利尿薬治療群よりもARBロサルタン＋利尿薬治療群のほうで糖尿病発生が少なかった[13]。この研究は最近発表になったVALUE研究と異なり，糖尿病発症がエンドポイントして設定され，診断基準なども事前に設けられ，毎年の検査により発症リスクがきちんとカプランマイヤーからのhazard ratioにて表現されているため信頼性も高い。またこの研究は，言ってみれば利尿薬と組み合わせるにはARBのほうが糖代謝についてはメリットがあると読み替えることもできる（もちろん重要な所見である）。この研究が優れているのは，薬剤の影響を過大評価せず，他の糖尿病発症のリスクと，β遮断薬群への割り付けによるリスクを比較して論じていることである[14]。それによると，いわゆる古典的なリスクおよび血圧がより重要とされる。その薬剤のプロモーションばかりにとらわれず，このような科学的なRCTの解釈こそ臨床の現場に貢献することは強調されるべきである。

日本ではトリクロルメチアジドとニカルジピンとの比較試験が行われたが[15]，この試験も厳密な単独治療の比較であり，トリクロルメチアジドが4 mgまで40%の患者において増量されている。結果として新たな糖尿病は利尿薬群で210名中4名に発生し，ニカルジピン群では発生がなかったが，できればより多い対象者において，降圧利尿薬は少量併用のかたちで比較すべきであると考えられる。表に降圧利尿薬を用いた臨床試験における糖尿病の発生についてまとめた。いずれも降圧利尿薬を含む治療群で糖尿病の発生が多い傾向にはあるが，今後低用量かつより適切な併用薬の使用のもとに評価すべきである。

図3に最近われわれが開始した研究のプロトコルを示す。この研究は本態性高血圧患者を低用量降圧利尿薬使用群（トリクロルメチアジドやインダパミドで1 mg/day以下，併用薬はいずれの降圧薬も可）非使用群（利尿薬以外いかなる降圧薬も可）にランダムに割り付け，一次エンドポイントとして糖尿病の発症を比較するものである。これまでの試験と異なりβ遮断薬＋利尿薬という併用ではなくARBやACE阻害薬も併用でき，しかも利尿薬の用量は厳格に低用量を維持する。このような使用法であれば，われわれは糖尿病の発生において差がないと考えており，したがって（そのような副作用の問題がなければ）医療経済学的にも安価な利尿薬の使用は有利なはずである。

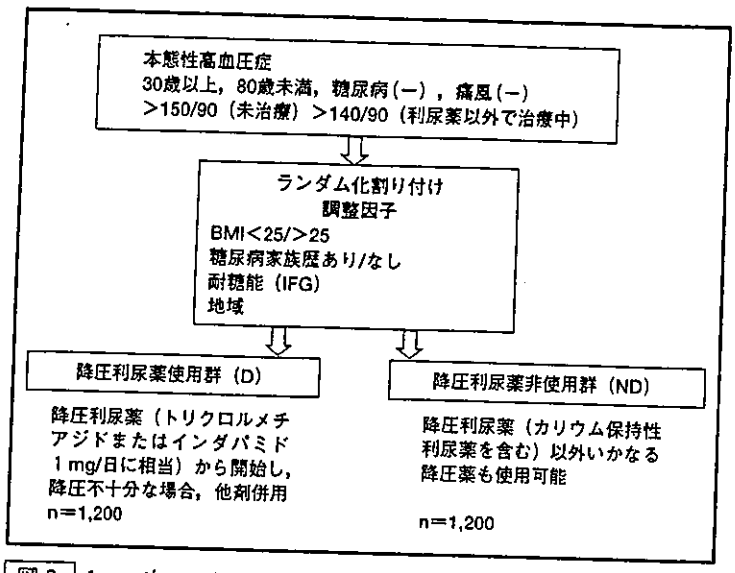

図3 1．一次エンドポイント：新たな糖尿病の発生
2．二次エンドポイント：①その他の有害事象，②血圧，血糖値，耐糖能，血中脂質，③心血管イベント
3．費用対効果の検討

■Ⅲ 降圧薬の糖代謝への影響が降圧作用を超えて心血管リスクに影響するか

高血圧そのものが糖尿病発生のリスクとなるならば，治療中の高血圧患者が糖尿病を発症したとしてもそれが降圧薬によるものか否かは明らかではない。もちろん中止して糖代謝が改善すればその可能性があるものの，それにしても全くの薬剤性糖尿病か，なんらかの代謝異常（たとえば高用量の降圧利尿薬による低カリウム血症）により糖尿病発症の素因ももつものがより早期に発症したものかは不明である。高血圧治療中の糖尿病発症の多くは自然経過としての発症であることが大規模なコホート研究からも示唆されている。したがってこれらの自然発症した糖尿病が将来，心血管リスクになりうることはある程度自明であるが，問題はある薬剤で糖尿病の発症が一部促進されたとして，そこが心血管リスクとなりうるかである。あるいは降圧が心血管リスク減少に働いている場合，同様に薬理作用としての軽微な代謝への影響をどう臨床的に判断するかである。

これはまず対象となる疾患のリスクがどの程度かによる。β遮断薬は冠動脈疾患，心筋梗塞患者の予後，心不全患者の生命予後を改善することが確認されている。このようなハイリスク患者群ではβ遮断薬によるリスク軽減が大きいため，おそらく糖代謝への影響は無視できるであろう。UKPDS研究ではハイリスクの糖尿病を伴う高血圧患者において厳格な血圧の管理により，糖代謝への影響という点からはβ遮断薬よりも有利なACE阻害薬カプトプリルと同等のリスク低下が得られている[16]。しかし，たとえば一次予防を目的とした高齢者高血圧の治療にはβ遮断薬が他剤ほど心血管リスク軽減に貢献しないと報告されており，そのような場合糖代謝の悪化が存在すれば，β遮断薬の使用にこだわる理由はない。もう1つの観点は併用薬である。LIFE研究のサブ解析の結果は，糖尿病をもつ高血圧患者において降圧利尿薬が使用されていれば，併用薬としてはβ遮断薬に比べARBロサルタンの方が糖代謝および生命予後の点

で有利であることを示唆している[17]。UKPDS研究との差は併用薬および心筋梗塞のリスク（UKPDSがよりリスクが高い）であろう。

降圧利尿薬の場合は糖尿病を伴う高血圧においてすらカルシウム拮抗薬と同等のリスク低下が得られていることや[18]，ACE阻害薬やARBは適切な降圧を達成するためには降圧利尿薬併用がほぼ必須であり（LIFE研究では9割が服用），目標血圧のより低い糖尿病においてはむしろ必要とされる場合が多く，糖代謝への影響は，基本的には個々の症例で判断すべきことではあるが，投与を思いとどまる理由にはならないと考えられる。逆にVALUE研究ではバルサルタンに降圧利尿薬を併用するタイミングが（倍量より降圧利尿薬追加のほうがより降圧するというデータがあるにもかかわらず）遅れたため血圧のコントロールの点で劣り，結果的に心筋梗塞と脳卒中が対照薬のアムロジピンよりも多いという結果になった[19]。また，PROGRESS研究では降圧利尿薬インダパミドとACE阻害薬ペリンドプリルの併用であれば脳卒中二次予防が可能であるが，ペリンドプリルエルブミン単独では不可能であった[20]。すなわちARBやACE阻害薬への利尿薬の追加は糖代謝においてはやや不利になるが，心血管リスクの軽減に関しては明らかに有益ではないだろうか。

しかし注意したいのは，最近の臨床試験は基本的に高リスク患者において行われており，投与期間が長期に及ぶ低リスク患者のデータはMRC研究以降存在しないことである。どちらかと言えば高リスク患者において投与されることの多いβ遮断薬と異なり，降圧利尿薬は低リスク患者に長期にわたって投与される可能性が高いので，低用量であれば糖代謝への影響が軽微であることを日本人高血圧患者において確認しなければならない。これだけのエビデンスがあるにもかかわらず，おそらく糖代謝悪化への懸念が日本における利尿薬処方の減少につながっていると思われる。またβ遮断薬と利尿薬は安価であり，適切に使用することによって安価に心血管イベントを減少させうることも忘れてはならない。糖代謝に拘泥するあまり，これらを使うべき患者に使用せず，結果として心血管リスクを減少させられないようなことは避けるべきである。

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Hypoadiponectinemia Is Closely Linked to Endothelial Dysfunction in Man

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Vascular endothelial dysfunction has been demonstrated in overweight or obese patients, but the molecular basis for this link has not been clarified. We asked what the relationship was between adiponectin, an adipose-specific molecule, and endothelial function. Forearm blood flow (FBF) was measured during reactive hyperemia by using strain-gauge plethysmography in 76 Japanese subjects without a history of cardiovascular or cerebrovascular disease, diabetes mellitus, hepatic, or renal disease. The peak FBF and total reactive hyperemic flow [flow debt repayment (FDR)] during reactive hyperemia were correlated with waist circumference ($r = -0.418$ and -0.414, respectively) and body mass index ($r = -0.597$ and -0.626, respectively). After correcting for age, gender, and body mass index, the peak FBF was correlated with systolic blood pressure ($r = -0.294$; $P = 0.010$), free fatty acid (FFA) ($r = -0.331$; $P = 0.004$), and adiponectin in log 10 ($r = 0.492$; $P < 0.001$), and FDR was correlated with adiponectin in log 10 ($r = 0.462$; $P = 0.001$). In stepwise multiple regression analyses, predictive variables for peak FBF were adiponectin in log 10 ($r = 0.468$) and FFA ($r = -0.292$; $r^2 = 0.487$; $P < 0.0001$); and predictive variables for FDR were adiponectin in log 10 ($r = 0.474$) and FFA ($r = -0.275$; $r^2 = 0.346$, $P < 0.0001$). Endothelial function was impaired in proportion to the severity of obesity, and the level of severity was closely related to plasma adiponectin levels. Adiponectin may play a protective role against the atherosclerotic vascular change, and loss of effects enhances endothelial dysfunction, as in obese people. (*J Clin Endocrinol Metab* 88: 3236–3240, 2003)

OBESITY IS ASSOCIATED with increased cardiovascular morbidity and mortality (1, 2). The American Heart Association determined that obesity is a major, modifiable risk factor for coronary heart disease, based on emerging data about the link between adiposity and coronary heart disease (3). Vascular endothelial dysfunction plays a pivotal role in the pathogenesis of atherosclerosis and enhances the risk of future cardiovascular events (4, 5). The presence of vascular endothelial dysfunction has been demonstrated in overweight or obese patients with insulin resistance (6, 7) and visceral obesity (8). However, the molecular basis for the link between obesity and vascular endothelial dysfunction has not been clarified. A newly discovered adipose-specific molecule, adiponectin, was shown to be decreased in obese people (hypoadiponectinemia) (9), as in patients with coronary artery disease (10). Because adiponectin may protect the endothelium from early atherosclerotic events such as the expression of adhesion molecules (10) or the attachment of monocytic cells (11), hypoadiponectinemia could be linked to endothelial damage. The present study questioned the relationship between plasma adiponectin levels and endothelial function in humans.

Abbreviations: BMI, Body mass index; FBF, Forearm blood flow; FDR, flow debt repayment; FFA, free fatty acid; HOMA-IR, homeostasis model assessment of insulin resistance; NO, nitric oxide; NTG, nitroglycerin; RH, reactive hyperemia.

Subjects and Methods

Subjects

This study consisted of 76 Japanese subjects without a history of cardiovascular or cerebrovascular disease, diabetes mellitus, hepatic, or renal disease. The study protocol complied with the Guidelines of the Ethical Committee of the University of the Ryukyus. Informed consent was obtained from all subjects.

Endothelial function

Forearm blood flow (FBF) was measured using a mercury-filled SILASTIC strain-gauge plethysmograph (EC-5R, D. E. Hokanson, Inc., Issaquah, WA), as previously described (12, 13). The strain gauge was attached to the upper arm, held above the right atrium, and connected to a plethysmographic device. A wrist cuff was inflated to a pressure of 200 mm Hg to exclude the hand circulation from the measurements 1 min before each measurement and throughout the measurement of FBF. The upper arm cuff was inflated to 40 mm Hg for 7 sec in each 15-sec cycle to occlude venous outflow from the arm, using a rapid cuff inflator (EC-20, D. E. Hokanson, Inc.). The FBF output signal was transmitted to a recorder (U-228, Advance Co., Nagoya, Japan). FBF was expressed as milliliters per minute per 100 ml of forearm tissue. The FBF was then calculated by two independent observers who had no knowledge of the subjects' profiles; the interobserver coefficient of variation was 3.0 ± 1.3%.

Study protocol

The study began at 0900 h, after the subjects fasted for at least 12 h. The subjects were kept in a supine position, in a quiet, dark, air-conditioned room (constant temperature of 25 C) throughout the study. After 30 min in the supine position, the basal FBF was measured. Then the effect of reactive hyperemia (RH) and sublingual nitroglycerin (NTG) on FBF was measured, as described, with modifications (14, 15).

To induce RH, FBF was occluded by inflating the cuff on the right upper arm to a pressure of 200 mm Hg for 5 min. After releasing the cuff, FBF was measured for 180 sec. Subjects were then given 0.3 mg of NTG sublingually, and FBF was measured for 5 min. The end of the response to RH or sublingual NTG was followed by a 15-min recovery period. Baseline blood samples were obtained after 30 min at rest. The peak FBF response (12) and total reactive hyperemic flow [flow debt repayment (FDR)] (16) during RH were used to assess the resistance of vessel endothelial function. FDR was defined as the curve under the area flow *vs.* time during RH above baseline flow (16). Because FDR, but not peak FBF, was significantly decreased by intraarterial infusion of N^G-monomethyl-L-arginine (4 μmol/min), a blocker of nitric oxide (NO) synthesis, we used FDR as a relatively NO-dependent marker (16). In the preliminary study, we confirmed the reproducibility of RH and sublingual NTG-induced vasodilation on two separate occasions in 28 healthy male subjects (mean age, 27 ± 5 yr). The coefficients of variation were 4.3% and 2.8%, respectively.

Biochemical measurements

Venous blood samples were obtained in tubes containing EDTA-sodium (1 mg/ml) and polystyrene tubes without an anticoagulant. The EDTA-containing tubes were promptly chilled. Plasma was immediately separated by centrifugation at 3000 rpm at 4 C for 10 min, and serum was separated by centrifugation at 1000 rpm at room temperature for 10 min. Samples were stored at −80 C until assayed. Routine chemical methods were used to determine the serum concentrations of total cholesterol, high-density lipoprotein cholesterol, triglycerides, creatinine, glucose, and electrolytes. The serum concentration of low-density lipoprotein was estimated using Friedewald's method (17). The plasma adiponectin concentration was measured by sandwich ELISA, as previously described (9). Homeostasis model assessment of insulin resistance (HOMA-IR), an insulin resistance index, was calculated as described (18).

Statistical analysis

Values are expressed as the mean ± SD. Comparisons of time-course curves of FBF during RH were analyzed by two-way ANOVA for repeated measures on one factor, followed by Fisher's protected least significant difference for multiple-paired comparisons. The repeated factor was time of RH, and the nonrepeated factor was one group *vs.* the other group. Multigroup comparisons of variables were made by one-way ANOVA followed by Fisher's protected least significant difference for multiple-paired comparisons. Probabilities less than 0.05 were considered to be significant. The data were processed using StatView J-5.0 software (SAS Institute Inc., Cary, NC).

Results

The mean values (range) of basal FBF, peak FBF, and the maximal flow after NTG administration (in milliliters per minute per 100 ml) were 3.27 ± 1.43 (1.37–6.19), 18.45 ± 6.76 (4.05–35.3), and 4.26 ± 1.64 (2.35–6.88) in all 76 subjects. Single correlation analyses showed that the peak FBF was negatively correlated with waist circumference, body mass index (BMI), systolic blood pressure, free fatty acid (FFA), and leptin, and was positively correlated with adiponectin (Fig. 1 and Table 1). FDR correlated negatively with waist circumference, BMI, HOMA-IR, FFA, and leptin, and positively with adiponectin. The maximal flow after NTG did not correlate with any metabolic and anthropometric variables. After correcting for age, gender, and BMI, the peak FBF was correlated only with systolic blood pressure, FFA, and adiponectin, and FDR was correlated with adiponectin. In stepwise multiple regression analyses, predictive variables for peak FBF were adiponectin in log 10 ($r = 0.468$) and FFA ($r =$

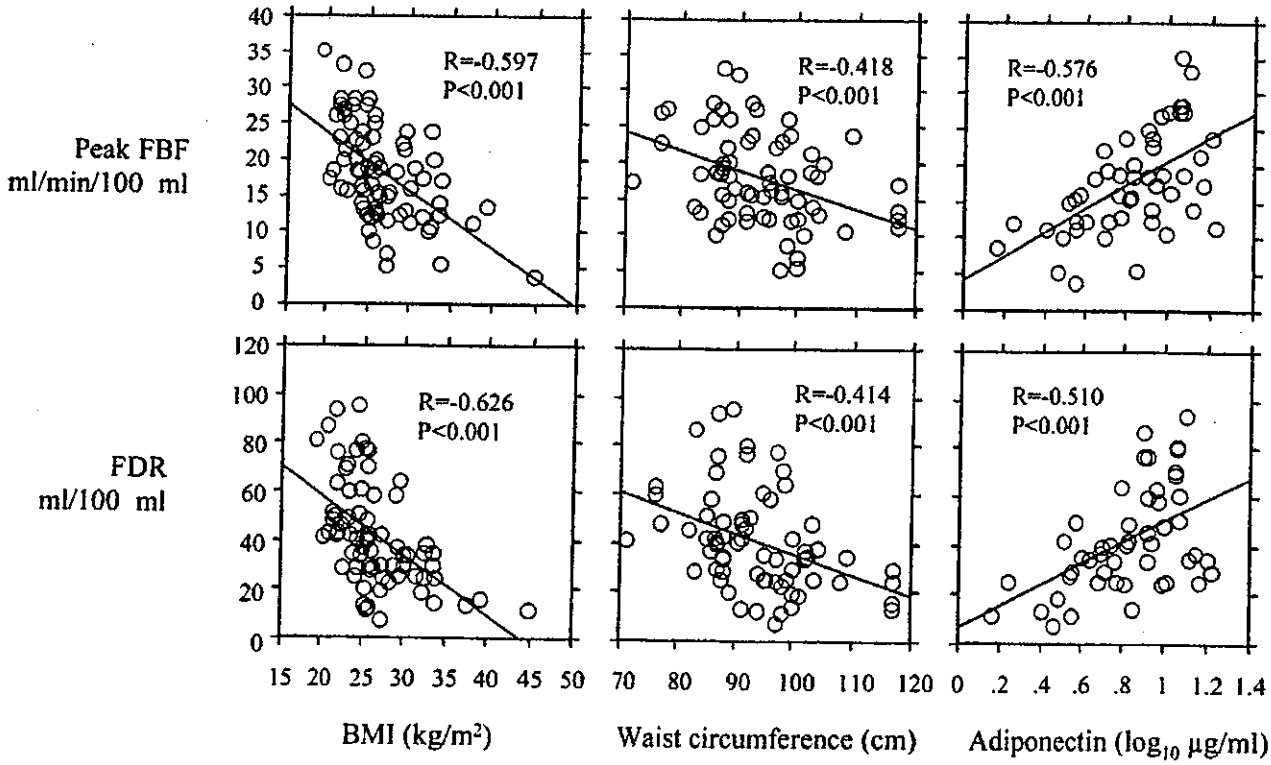

FIG. 1. Correlation between peak FBF (*upper panels*) and FDR (*lower panels*) during RH, BMI, waist circumference, and serum adiponectin levels in all 76 subjects. Pearson's correlation coefficients are shown.

TABLE 1. Peak FBF, FDR, and variables

	Peak FBF				FDR			
	Simple correlation		Partial correlation		Simple correlation		Partial correlation	
	r[a]	P	r[b]	P	r[a]	P	r[b]	P
Age (yr)	−0.075	0.609			−0.177	0.215		
Waist circumference (cm)	−0.418	0.000	0.088	0.469	−0.414	0.000	0.090	0.455
BMI (kg/m^2)	−0.597	<0.0001			−0.626	<0.0001		
Systolic blood pressure (mm Hg)	−0.304	0.008	−0.294	0.010	−0.201	0.082	−0.118	0.310
Hemoglobin A1c	−0.156	0.194	0.047	0.697	−0.230	0.052	−0.023	0.848
HOMA-IR	−0.206	0.090	−0.021	0.864	−0.269	0.024	−0.052	0.669
Triglyceride (mg/100 ml)	−0.033	0.780	−0.081	0.493	−0.046	0.696	−0.016	0.892
LDL cholesterol (mg/100 ml)	−0.082	0.494	−0.124	0.296	−0.034	0.775	−0.129	0.273
FFA (mmol/liter)	−0.386	0.001	−0.331	0.004	−0.336	0.003	−0.210	0.073
Leptin ($\log_{10}$ ng/ml)	−0.453	0.000	−0.008	0.950	−0.349	0.004	0.019	0.880
Adiponectin ($\log_{10}$ μg/ml)	0.576	<0.0001	0.492	0.000	0.510	<0.0001	0.462	0.001

LDL, Low-density lipoprotein.
[a] r, Pearson's correlation coefficient.
[b] r, Variables corrected by age, gender, and BMI.

TABLE 2. Stepwise multiple regression models predicting peak FBF and FDR

	Peak FBF				FDR			
	Model 1		Model 2		Model 1		Model 2	
	β	F	β	F	β	F	β	F
Adiponectin ($\log_{10}$ μg/ml)	0.468	16.473	0.439	16.907	0.474	14.354	0.388	12.58
Age (yr)	0.084	0.287	−0.122	0.621	−0.125	0.662	−0.281	3.59
Gender	0.258	2.857			0.012	0.006	−0.016	0.01
BMI (kg/m^2)			−0.498	21.765			−0.516	22.25
HOMA-IR	−0.186	1.439	0.06	0.148	−0.063	0.170	0.225	2.24
LDL cholesterol (mg/100 ml)	0.044	0.077	−0.093	0.358	−0.069	0.202	−0.144	0.88
FFA (mmol/liter)	−0.292	6.609	−0.274	3.317	−0.275	4.817	−0.208	1.90
Systolic blood pressure (mm Hg)	−0.273	5.75	−0.286	3.664	−0.192	1.599	−0.101	0.44
Hemoglobin A1c	−0.268	3.096	−0.149	0.93	−0.236	2.482	−0.184	1.47
r		0.698		0.745		0.588		0.721
r^2		0.487		0.555		0.346		0.520
F		12.976		26.151		11.353		23.33
P		<0.0001		<0.0001		<0.0001		<0.0001

The F value to enter was 4.0 at each step. β, Parameter estimate; LDL, low-density lipoprotein.

−0.292; r^2 = 0.487; $P < 0.0001$), and predictive variables for FDR were adiponectin in log 10 (r = 0.474) and FFA (r = −0.275; r^2 = 0.346; $P < 0.0001$) (Table 2, model 1). When BMI was included in the model (Table 2, model 2), predictive variables for peak FBF and FDR were adiponectin in log 10 (r = 0.439 and 0.388, respectively) and BMI (r = −0.498 and −0.516, respectively). Serum adiponectin in log 10 (micrograms per milliliter) was negatively correlated with waist circumference (r = −0.334; P = 0.020), BMI (r = −0.365; P = 0.007), HOMA-IR (r = −302; P = 0.034), and FFA (r = −0.271; P = 0.052).

Discussion

The major findings of the present study were: 1) the peak FBF response and FDR to RH, indices of the function of vessel endothelial resistance, were impaired in proportion to the severity of obesity; and 2) the impairment in the FBF response and FDR was correlated with low levels of serum adiponectin. This is the first study that shows the effects of adiponectin on endothelial function in human subjects and that low levels of adiponectin were closely correlated with resistance vessel endothelial dysfunction.

Serum adiponectin and endothelial function

Measurements of FBF during intraarterial infusion of acetylcholine are used to investigate endothelium-dependent vasodilatation (12, 13). However, this technique is invasive and time consuming and cannot be used routinely. We measured FBF during RH using strain-gauge plethysmography. With this noninvasive method, resistant vessel endothelial function can be assessed physiologically with a high reproducibility (14, 15). Higashi *et al.* (15) reported that peak FBF during RH was well correlated with FBF to maximal acetylcholine dose (30 μg/min; r = 0.91; $P < 001$), indicating that this noninvasive method is a useful alternative for assessing resistance vessel endothelial function. Because the contribution of NO may be different at the RH phase, we used peak FBF as a combination marker of shear stress and local metabolic factors at an early phase of RH, and FDR as a relatively NO-dependent marker at the mid-to-late phase of RH (16).

The peak FBF and FDR were correlated negatively with waist circumference, BMI, FFA, systolic blood pressure (peak FBF), HOMA-IR (FDR), and leptin, and correlated positively with adiponectin. After correcting for age, gender, and BMI, the endothelial function indices were correlated positively

with adiponectin and negatively with FFA. NTG-induced FBF changes were not correlated with any metabolic and anthropometric variables, indicating that endothelium-dependent function was predominantly affected by hypoadiponectinemia. It is suggested that, in humans, the plasma level of adiponectin may be directly linked to endothelial function.

Mechanisms for a link between hypoadiponectinemia and endothelial dysfunction

Two possible mechanisms by which hypoadiponectinemia decreases endothelial function are postulated.

First, adiponectin level can be linked to whole-body insulin sensitivity, and hypoadiponectinemia can cause endothelial dysfunction by decreasing insulin sensitivity. Because the plasma adiponectin level was decreased in the prediabetic insulin-resistant phase in rhesus monkeys (*Macaca mulatta*), hypoadiponectinemia might play a causative role in the development of insulin resistance (19). We confirmed this concept by showing that experimental ablation of the adiponectin gene in mice reduces insulin sensitivity, and adenovirus-mediated supplementation of plasma adiponectin can recover the sensitivity (20). There is a close correlation between whole-body insulin sensitivity and endothelium-dependent vasodilatation (6, 7). We previously reported that endothelial NO production (21) and vasodilatation induced by local intraarterial infusion of insulin/glucose (22) were both closely linked to whole-body insulin sensitivity (21). In the current study, FDR was negatively correlated with HOMA-IR (Table 1), indicating a link between FDR and insulin sensitivity. But this correlation was abolished after correcting for age, gender, and BMI, probably by a strong confounding effect of BMI on insulin sensitivity. In multiple regression analysis, endothelial function was negatively correlated with FFA and positively correlated with adiponectin (Table 2, model 1). When BMI was included in the model as an independent variable, the power of FFA, but not of adiponectin, was abolished (Table 2, model 2). HOMA-IR, a casual marker for insulin resistance (18), was not selected as a predictor for endothelial function. Serum adiponectin levels were reported to be closely linked to insulin sensitivity in human subjects (19, 23), and the current study showed that adiponectin levels were correlated negatively with waist circumference, BMI, and HOMA-IR, indicating a close link between hypoadiponectinemia and insulin resistance. Adiponectin might eliminate the predictive power of HOMA-IR, which is a relatively weaker predictor for insulin sensitivity than other markers, such as an M-value by the hyperinsulinemic euglycemic clamp (23). It was also reported that FFA can directly impair endothelial function in humans by decreasing insulin sensitivity (6, 7). Adiponectin (19, 29, 23) and FFA (6, 7), which are both secreted from adipocytes, may independently and bidirectionally regulate endothelial function through modulation of insulin sensitivity in the whole body and vascular beds.

Second, hypoadiponectinemia may be directly linked to early atherosclerotic vascular damage and a subsequent endothelial dysfunction. Experimentally, Ouchi *et al.* (10) showed that adiponectin inhibited TNF-α-induced expression of endothelial adhesion molecules in endothelial cells and that adiponectin reduced atherogenic transformation of macrophage to foam cells by suppressing scavenger receptor expression (11). It was also evident that plasma adiponectin levels were decreased in patients with atherosclerotic risk factors such as obesity, impaired glucose tolerance, diabetes mellitus, or previous coronary heart disease (10). Loss of plasma adiponectin may accelerate early atherosclerotic vascular damage and reduce various physiological roles of endothelial cells, including NO synthesis and supply, which may be linked to decreases in the peak FBF and FDR, in the current study.

Study limitation

The current study cannot determine which of the following is plausible: 1) hypoadiponectinemia impairs endothelial function by impairment of insulin sensitivity in the whole body and vascular beds; 2) hypoadiponectinemia first accelerates vascular damage and then impairs NO supply from endothelium; or 3) hypoadiponectinemia first impairs NO synthesis and supply and then accelerates vascular damage (3). Direct actions of adiponectin on vascular function should be observed in future studies.

Conclusions

The current study showed that endothelial function was impaired in proportion to the severity of obesity and that endothelial function was closely related to plasma adiponectin levels. Adiponectin may play a protective role directly against the atherosclerotic vascular change and/or indirectly through improving insulin sensitivity. The loss of adiponectin effects enhances endothelial dysfunction and may be associated with future cardiovascular events.

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A single dose of nateglinide improves post-challenge glucose metabolism and endothelial dysfunction in Type 2 diabetic patients

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Abstract

Aims This randomized crossover placebo-controlled study aimed to assess the efficacy of nateglinide, a phenylalanine-derived insulin secretagogue, on forearm endothelial function in diabetic subjects before and after an oral glucose load.

Methods Forearm blood flow (FBF) was measured using strain-gauge plethysmography during reactive hyperaemia before and after an oral glucose load (75 g) with a prior use of placebo or nateglinide (90 mg) in 15 diet-treated Type 2 diabetic patients or age-matched controls with normal glucose tolerance.

Results The peak FBF response and total reactive hyperaemic flow (flow debt repayment: FDR), indices of resistance artery endothelial function, were decreased after an oral glucose load in diabetic patients, but unchanged in controls. Nateglinide administered to diabetic patients accelerated insulin secretion and reduced post-challenge plasma glucose, and also abolished the post-challenge impairment of endothelial function. The peak FBF and FDR were well correlated with 120-min glucose levels and 30-min insulinogenic index.

Conclusions A single challenge of glucose was shown to impair endothelial function in diabetic patients, and the post-challenge endothelial dysfunction was improved by a prior use of nateglinide. Long-term effects of nateglinide on endothelial function in Type 2 diabetic patients need to be clarified in future studies.

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Keywords endothelial function, nateglinide, post-challenge glucose metabolism, Type 2 diabetes

Introduction

Post-prandial hyperglycaemia has been associated with increased risk of macrovascular complications [1–3]. A meta-analysis including 95 783 people from 22 studies demonstrated the association between post-challenge hyperglycaemia and cardiovascular events [1]. Analysis of the prospective DECODE (the Diabetes Epidemiology: Collaborative Analysis of Diagnostic Criteria in Europe) data [2] and the Honolulu Heart Program [3] showed that elevated 2-h glucose was a better predictor of mortality from cardiovascular diseases than fasting glucose alone. In a screened multicentre cohort, the Diabetes Intervention Study showed that poor control of post-prandial glucose increased the risk of myocardial infarction or mortality up to 3-fold, even under good control of fasting glycaemia [4]. Vascular endothelial dysfunction plays a pivotal role in the pathogenesis of atherosclerosis [5] and enhances the risk of future cardiovascular events [6,7]. The presence of vascular endothelial dysfunction has been demonstrated in Type 2 diabetic patients [8], but the contributions of post-prandial hyperglycaemia to endothelial dysfunction is largely unknown [9]. This crossover placebo-controlled study aimed to assess the efficacy of post-challenge hyperglycaemia reduction by nateglinide, which

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rapidly enhances insulin secretion and reduces mealtime glucose excursions [10], on forearm endothelial function in Type 2 diabetic subjects.

Research design and methods

Diet-treated Type 2 diabetic patients (six male and nine female, 60 ± 3 years) without a history of cardiovascular complication were enrolled in this study. Age-matched healthy subjects with normal glucose tolerance were used as controls. The study protocol was approved by the Ethical Committee of the University of the Ryukyus and carried out in accordance with the principles of the Declaration of Helsinki. All subjects gave informed consent. No subjects were taking medication, and all abstained from alcohol, tobacco and strenuous physical activity for 24 h and caffeine-containing drinks overnight.

The acute effects of oral glucose loading with placebo or nateglinide on forearm blood flow (FBF) was studied in a double-blinded placebo-controlled crossover design. Studies were done on two separate mornings at least 1 week apart. After overnight fasting, either 90 mg nateglinide (Yamanouchi Pharmaceutical, Tokyo, Japan) or placebo was ingested, followed by an oral 75-g glucose load (Trelan-G, Takeda, Japan). We measured FBF during reactive hyperaemia using a mercury-filled silastic strain-gauge plethysmograph (EC-5R, D. E. Hokanson Inc., Issaquah, WA, USA) before and 120 min after a glucose load [11–13]. Before and after release of a 5-min upper arm cuff occlusion at 200 mmHg (reactive hyperaemia), FBF was measured by repeated inflations of the upper arm at 40 mmHg during a wrist cuff inflation at 200 mmHg [11,12]. We used peak FBF as a combination marker of shear stress and local metabolic factors at early phase of RH, and total reactive hyperaemic flow (flow debt repayment: FDR), as a relatively NO-dependent marker at mid-to-late phase of RH [13].

Values are expressed as the mean ± SEM. Two-tailed unpaired Student's *t*-test and two-way analysis of variance (ANOVA) for repeated measures on one factor followed by Bonferroni's correction for multiple-paired comparisons, were analysed using StatView J-5.0 (SAS Institute, Cary, NC, USA) software package.

Results

The baseline characteristics of the 15 diabetic subjects are as follows: body mass index 26.1 ± 1.13 (kg/m^2); heart rate 73 ± 3 (beats/min); blood pressure 129/72 ± 4/2 (mmHg); total cholesterol 5.39 ± 0.20 (mmol/l); triglyceride 1.63 ± 0.21 (mmol/l); HDL-cholesterol 1.42 ± 0.05 (mmol/l); and glycosylated haemoglobin A_{1c} 7.53 ± 0.30 (%).

With a prior use of placebo, plasma insulin levels were low at 30 min and peaked at ≈ 350 pmol/l between 90 and 120 min after an oral glucose load, and plasma glucose levels remained ≈ 19 mmol/l at 120 min (Table 1). With a prior use of nateglinide, post-challenge insulin levels increased rapidly at 30 min and glucose levels significantly decreased to ≈ 15 mmol/l at 120 min. All medications were well tolerated and no adverse events were observed during the study. Systemic haemodynamics and metabolic parameters at baseline were comparable between two study days.

Table 1 Plasma glucose and serum insulin levels after an oral glucose load during a prior use of placebo or 90 mg nateglinide in 15 diabetic patients

Time (min)	Pasma glucose (mmol/l)		Serum insulin (pmol/l)	
	Placebo	Nateglinide	Placebo	Nateglinide
0	8.8 ± 0.4	8.2 ± 0.4	85 ± 14	64 ± 11
30	14.6 ± 0.6	13.1 ± 0.5	198 ± 19	345 ± 25
60	18.1 ± 0.8	14.9 ± 0.8	309 ± 37	351 ± 51
90	19.4 ± 0.6	15.7 ± 0.8	358 ± 51	403 ± 60
120	19.2 ± 0.8	14.9 ± 0.6	350 ± 45	431 ± 70
P-value	$P < 0.001$		$P = 0.056$	

Data represent the mean ± SEM. The *P*-values for curve difference by two-way ANOVA were shown.

With placebo, FBF response during reactive hyperaemia decreased significantly after a glucose load ($P < 0.001$ for trend), but, with nateglinide, post-challenge FBF response remained unchanged ($P = 0.137$). The peak FBF response and FDR, indices of resistance artery endothelial function, were both decreased after an oral glucose load in diabetic patients, but not in healthy controls (Fig. 1a). A single ingestion of nateglinide prevented the post-challenge decrease in the peak FBF and FDR. The peak FBF and FDR were well correlated with 120-min glucose levels (Fig. 1b). Peak FBF was negatively correlated with area under the curve for glucose ($AUC_{glucose}$, $R = -0.423$, $P = 0.017$) and insulin ($AUC_{insulin}$, $R = -0.387$, $P = 0.034$). Peak FBF and FDR were positively correlated with 30 min insulinogenic index (Δinsulin/Δglucose) ($R = 0.450$, $P = 0.012$ and $R = 0.438$, $P = 0.015$, respectively),

Discussion

This is the first study demonstrating that endothelial function was impaired by acute post-challenge glucose excursion in Type 2 diabetic patients, and a single dose administration of nateglinide abrogated the post-challenge endothelial dysfunction by decreasing the glucose excursion.

The peak FBF and FDR were both decreased after an oral glucose load in diabetic patients, but not in healthy controls with normal glucose tolerance. Kawano *et al.* [14] showed that endothelium-dependent flow-mediated dilatation (FMD) of the brachial artery were decreased after an oral glucose load in patients with Type 2 diabetes or impaired glucose tolerance. As there was a close negative correlation between indices of endothelial function and plasma glucose levels, post-prandial hyperglycaemia itself could be the culprit for post-prandial endothelial dysfunction [9,14]. Williams *et al.* [15] demonstrated that effects of acute hyperglycaemia on endothelium-dependent vasodilation were similar after blocking insulin release with octreotide, supporting this notion. A potential mechanism by which post-prandial hyperglycaemia impairs endothelial function could be generation of reactive oxygen stress

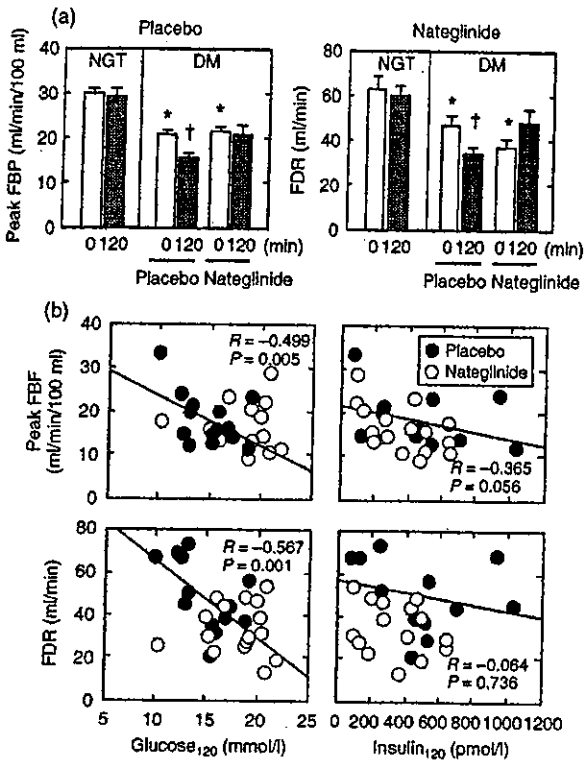

Figure 1 (a) Peak forearm blood flow (FBF) and flow debt repayment (FDR) before (0 min, open bars) and after (120 min, closed bars) an oral glucose load during a prior ingestion of placebo or nateglinide in 15 diabetic patients. For comparison, values in healthy subjects with normal glucose tolerance (NGT) were shown. Data represent the mean ± SEM. $*P < 0.05$ vs. NGT and $\dagger P < 0.05$ vs. 0 min. (b) Correlation between 120-min glucose and insulin levels and peak FBF and FDR after an oral glucose load in diabetic patients. An ingestion of either placebo (open circles) or 90 mg nateglinide (closed circles) was followed by an oral 75-g glucose load. Pearson's correlation coefficients (R) and P-values were shown.

(ROS) [9,14,16], as antioxidants can restore post-prandial endothelial function [17,18].

A single administration of nateglinide abrogated the post-challenge endothelial dysfunction in diabetic patients. As reported [10], nateglinide recovered early phase insulin secretion and decreased post-challenge glucose levels (Table 1). This raises the question whether the improvement of post-challenge endothelial function by nateglinide is the result of a suppression of post-challenge hyperglycaemia, a rise in insulin, or an effect of the drug itself. As 120-min glucose levels and $AUC_{glucose}$ were well correlated with peak FBF and FDR, suppression of post-challenge hyperglycaemia seemed to be the most likely factor. As peak FBF and FDR were negatively correlated with $AUC_{insulin}$, insulin resistance also might be involved in the endothelial dysfunction of diabetic patients. Nateglinide significantly increased insulinogenic index, but did not alter $AUC_{insulin}$ and 120-min insulin levels. This suggests that suppression of post-challenge hyperglycaemia by a recovery of early insulin secretion, but not an increase in insulin-mediated vasodilatation [19,20], explained the improved endothelial function.

In summary, a single glucose challenge impaired endothelial function in Type 2 diabetic patients, which was improved by prior use of nateglinide. Long-term effects of nateglinide on endothelial dysfunction in Type 2 diabetic patients need to be clarified in future studies.

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1 血管内皮機能診断法

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2型糖尿病においては，診断されたときはすでに動脈硬化は進行していることが知られている。これはpre-diabetic stateにおいてすでにインスリン感受性の低下とともに機能的動脈硬化ともいうべき血管内皮機能低下が存在するためと考えられる。また糖尿病患者における血管不全の病態を理解するには内皮機能低下とともに平滑筋の一酸化窒素（nitric oxide；NO）への反応低下も考慮されるべきである。これらの観点からアセチルコリンを用いた抵抗血管の内皮機能診断法，血流依存性血管拡張反応検査（flow mediated dilatation；FMD）を用いた伝導血管の内皮機能診断法について述べる。

アセチルコリン動注による血管拡張反応のプレシスモグラフによる測定

方法の概略

静脈閉塞プレシスモグラフはおよそ100年前にすでに報告されている前腕血流量測定法である[1]。原理は上腕にまいたカフを40mmHgに加圧することによって静脈還流を遮断し，動脈からの血液の流入量（＝前腕血流量）を前腕周の変化として計測する。現在手首に巻いたカフを200mmHgに加圧することによって手の血流を遮断して行われる。これは，前腕血流の30%を皮膚血流が占めるが，手の血流は特に温度に影響され，調節機構も骨格筋血流と異なるためである。また現在ではほぼその記録，計測もコンピュータ化され，より客観性が改善している。この方法による前腕血流量の測定に，brachial arteryからの薬物注入を組み合わせ，さまざまな*in vivo*の薬理学，生理学的な実験がヒトで行われている［1］。ほとんどの血管作動性物質は全身投与量の1/100～1/1,000で局所の血管収縮，拡張作用を引き起こすので，全身の血行動態に影響を及ぼすことなく，局所血管作用を評価できる。ヒト血管内皮機能はアセチルコリン（10～400nmol/min）を動注し，内皮依存性の血管拡張作用による前腕血流量の増加を測定することによって評価される［1，2］。また対照として主にニトロプルシドを動注し，内皮非依存性の血管拡張による前腕血流量の増加を測定する。この方法により血管平滑筋のNOへの反応性を評価することもできるので，アセチルコリンの血管拡張反応の低下が，内皮機能の低下か，NOへの反応性低下によるものか判定することができる。

解析方法

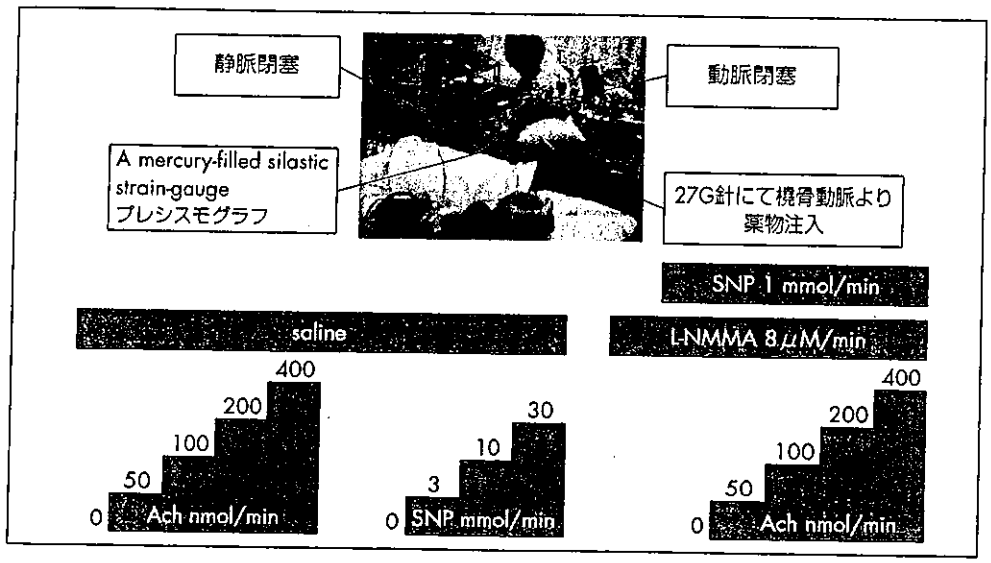
動注は通常左腕で行う（左利きの場合は右腕）が，筆者らは常に左右の血流量を測定してその比を算出している。したがって薬物の血管収縮または拡張作用はこの左右の血流量比で表される。これは非特異的な刺激による前腕血流量の変化を除外し，動注した薬物の効果をより正確に判定するためである[2]。

科学的妥当性は？

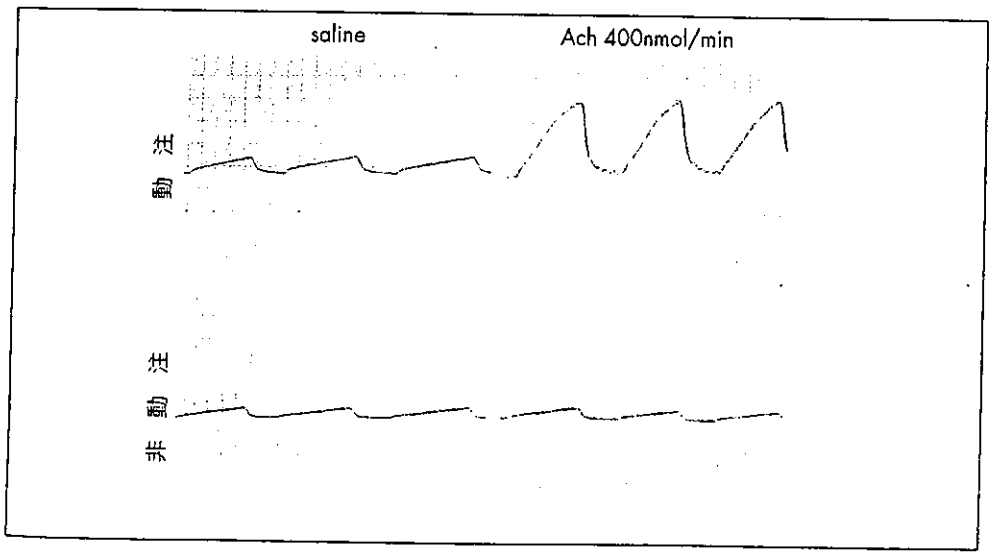
本法の科学的根拠はノーベル賞の対象となった1980年のFurchgottらの論文に求められる[3]。彼らは血管がアセチルコリンにより弛緩するには内皮細胞の存在が必要であり，このことからアセチルコリンは内皮細胞を刺激し血管拡張性物質［内皮依存性血管弛緩因子（endothelium derived relaxing factor；EDRF)］を遊離させることを提唱した。本法は後述するような方法論的あるいは解釈上の問題点を有するものの，内皮機能評価の標準法として十分な科学的妥当性をもつと考えられる。

方法論的妥当性—再現性とconfounding factors

プレシスモグラフによる前腕血流量の測定そのものの再現性は筆者らにより検討されている[2]。血流量の絶対値の再現性は良好とはいえず，左右の血流量の比はCV20%以下と比較的よい。前述したように筆者らは常に左右の血流比の変化を算出しているが，アンジオテンシンIIやノルアドレナリンの血管収縮作用に関しては血流量比のほうが再現性に優れる。アセチルコリンの血管拡張作用については再現性の観点からは両側測定の優位性は明らかではない。しかし基礎値の重要性を考慮すると理想的には両側測定を行うべきであろう。筆者の研究室ではアセチルコリン血管拡張反応の再現性（CV）は約15%である。再現性は本法を抗動脈硬化治療の



[1] 薬物の動注を併用したプレシスモグラフによる両側の前腕血流量測定
写真の下は標準的なNO依存拡張の測定を含めた内皮機能測定のための実験プロトコール。



[2] プレシスモグラフで測定された実際の前腕血流量とアセチルコリンによる増加