ment in clinical practice. Further large-scale studies are required for confirming the safety of catechin inhalation.

CONCLUSION

The catechin inhalation appeared to reduce the MRSA count in sputum. However, the application of catechin inhalation as a supplementary treatment for controlling MRSA infection remains controversial. Further studies are required for the evaluation of catechin inhalation effects on MRSA.

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スタチン治療における海外と国内のエビデンスの比較

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はじめに

3-hydroxy-3-methylglutaryl coenzyme A（HMG-CoA）還元酵素阻害薬（スタチン）は，強力なLDLコレステロール（LDL-C）低下作用を有することから高コレステロール血症治療の第一選択薬として世界中で広く使用されている．欧米における大規模臨床試験では，スタチンによるLDL-C値の低下と，心血管イベント発生率の減少が有意に相関することが示されてきた．

しかし，スタチン投与の有益性は対象とする患者の心血管イベントリスクに依存しており，日本人のように虚血性心疾患発症率が欧米に比べ低い対象集団におけるスタチンの有用性についてはこれまで不明であった．

2005年の米国心臓病学会で発表されたMEGA Studyの結果は上記の疑問に答えるものであり，本稿ではMEGA Studyの結果を中心に，スタチン治療における海外と国内のエビデンスを比較したい．

エビデンスを評価するために

海外と国内のエビデンスを比較する場合，エビデンスのもととなる臨床試験の目的，対象症例，試験方法，用いられた評価項目などを吟味することはもちろんだが，得られた結果についても相対リスク減少率（relative risk reduction: RRR），絶対リスク減少率(absolute risk reduction: ARR)やnumber needed to treat（NNT）など，さまざまな指標を用いて評価することが必要となる．これらの指標について，ベースラインのイベント発生率が異なる以下の2つのケースを例にあげて紹介したい．

ケース1：コントロール群でのイベント発生率は　50人/100人
介入（治療）を行うとイベント発生率が　30人/100人　に低下．

ケース2：コントロール群でのイベント発生率は　50人/10,000人
介入（治療）を行うとイベント発生率が　30人/10,000人　に低下．

RRRは，コントロール群と介入群とでリスクが相対的にどの程度減少したかを示す指標である．ケース1とケース2では，ベースラインのイベント発生率が100倍異なるにもかかわらず，RRRはともに40％となる（表1）．RRRで示した場合には，このようにベースラインリスクの差が反映されない．

ARRは，コントロール群でのイベント発生率と介入群でのイベント発生率の絶対的な差を示したもので，ケース1では20％であり，ケース2

[Key words] HMG-CoA reductase inhibitor, statin, clinical evidence, primary prevention, coronary heart disease, pleiotropic effects

表1　相対リスク減少率（RRR）

	コントロール群での イベント発生率	介入群での イベント発生率	RRR
ケース1	50人/100人	30人/100人	$\frac{50-30}{50}=0.4$（＝40%）
ケース2	50人/10,000人	30人/10,000人	$\frac{50-30}{50}=0.4$（＝40%）

表2　絶対リスク減少率（ARR）

	コントロール群での イベント発生率	介入群での イベント発生率	ARR
ケース1	50人/100人	30人/100人	$\frac{50}{100}-\frac{30}{100}=0.2$（＝20%）
ケース2	50人/10,000人	30人/10,000人	$\frac{50}{10,000}-\frac{30}{10,000}=0.002$（＝0.2%）

表3　一つのイベントを抑制するために介入（治療）しなければならない患者数（NNT）

	コントロール群での イベント発生率	介入群での イベント発生率	$NNT=\frac{1}{\text{絶対リスク減少率}}$
ケース1	50人/100人	30人/100人	$\frac{1}{0.2}=5$人
ケース2	50人/10,000人	30人/10,000人	$\frac{1}{0.002}=500$人

では0.2%となる（**表2**）．RRRで欠けていたベースラインリスクを内包した，より客観的な指標といえる．

NNTは，ARRの逆数をとったもので，一つのイベント発生を抑制するために介入（治療）しなければならない患者数を示している．ケース1では5人，ケース2では500人となる（**表3**）．ケース1では，きわめて効率の高い治療となるが，ケース2では，500人に治療を行ってはじめて一人のイベントを抑制できることが示され，逆に考えると499人にとっては治療しても，しなくてもアウトカムは変わらないことを意味している．ARRに比べ，より直感的に介入の重みを伝える点で優れており，医療経済学的な効果を検討するうえでも重要な指標とされる．このように同じ結果であっても，RRRで示されるか，ARRあるいはNNTで示されるかで，受け手側の印象は異なってくる．

多くの疫学的観察研究から血清総コレステロール値あるいはLDL-C値が上昇するに従い，男女を問わず海外でも国内でも冠動脈疾患発症リスクが増加することが示されてきた（**図1**）．この場合，海外と国内との類似点を示すためにしばしば用いられるのは，血清総コレステロール値に対応した冠動脈疾患発症の相対リスク変化であり，絶対リスクを指標とした場合には海外と国内で大きく異なることを認識しなければならない．

大規模研究からみた海外と国内の虚血性心疾患の絶対リスクの差

J-LIT試験は，日本人を対象とし約4万例，6

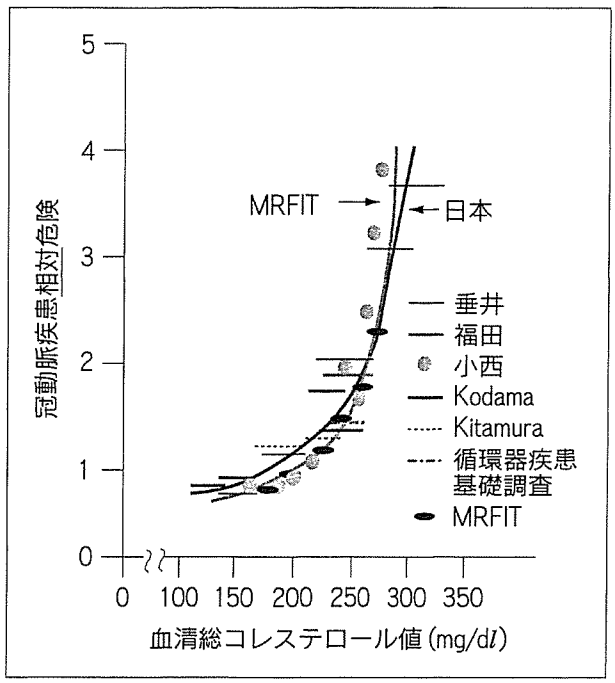

図1　血清総コレステロール値と冠動脈疾患相対危険：日本と米国の成績の対比

年間にわたる大規模観察研究であり，スタチン使用時の血清脂質値と虚血性心疾患の発症頻度に関して貴重な疫学的データを提供している[1]．総コレステロール値が220 mg/d*l* 以上の高脂血症患者を対象とした一次予防目的の観察研究であり，登録時の平均総コレステロール値は270±34 mg/d*l* と，同じく一次予防を目的としたWOSCOPSの登録時の平均総コレステロール値272±23 mg/d*l* に近似している[2),3)]．J-LITではプラセボ対照を欠き全例にsimvastatinが投与されているが，プライマリーエンドポイントである致死性・非致死性心筋梗塞，および心突然死の発生率は0.91/1,000人・年と，WOSCOPSの治療群（pravastatin投与群）の13.6/1,000人・年に比較して1/15近い低頻度であることが判明した．WOSCOPSは男性を対象とし，男女を含むJ-LITと直接比較することは不可能であるが，絶対リスクが海外と国内で大きく異なることは明らかである．

海外で行われた冠動脈疾患患者を対象とした二次予防試験では，スタチン投与によってLDL-Cレベルが低下するとともに，心血管イベント発生率や冠動脈疾患死亡率，さらに総死亡率が有意に低下することが示されている．また冠動脈疾患の既往歴のない患者を対象にした一次予防試験でも，スタチン投与により，心血管イベント発生率が低下し，冠動脈疾患死亡率や総死亡率も低下する傾向にあるが，その有益性は二次予防に比べ減少する．同じスタチンを使用した試験であっても一次予防試験と二次予防試験で有益性の程度が異なるのは，対象とした患者集団のリスクの程度が異なるからであり，一般的に薬物治療の利益は，対象とする患者のリスクの高低に依存している．このことはL'Abbe plotを用いると理解しやすい．

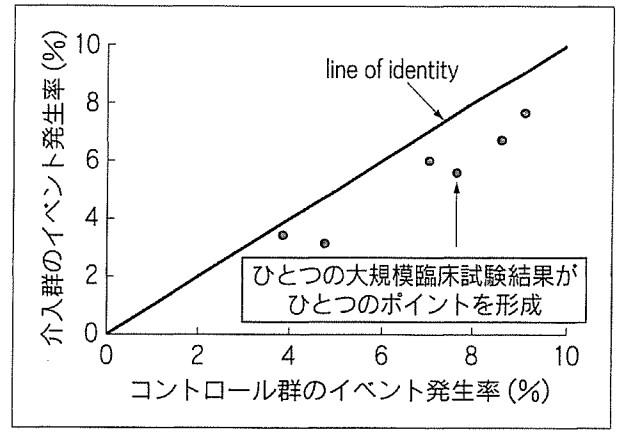

図2　L'Abbe Plot (1)

L'Abbe Plot解析による有益性と有害性の分岐点

L'Abbe Plotとは，*x*軸をコントロール（プラセボ）群のイベント発生率，*y*軸を介入群のイベント発生率とする直交座標系に，同じ目的の複数の試験結果をプロットしていき，それらを結んだ近似線が*y*=*x*の直線（line of identity：介入による効果が±0の基準線）の下にくるか上にくるかで，介入の有益性と有害性を見極めようとする手法である（図2）[4)]．薬物投与などの介入が有効である場合には，*y*<*x*領域にプロットされ，有害性が有益性を上回る場合には，*y*>*x*領域にプロットされることとなる．同じ目的で実施されたど

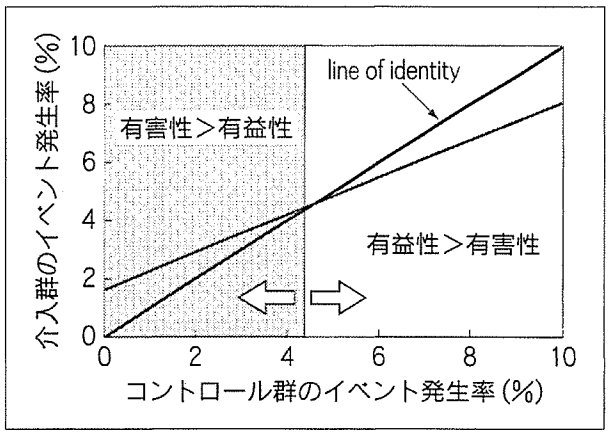

図3　L'Abbe Plot (2)

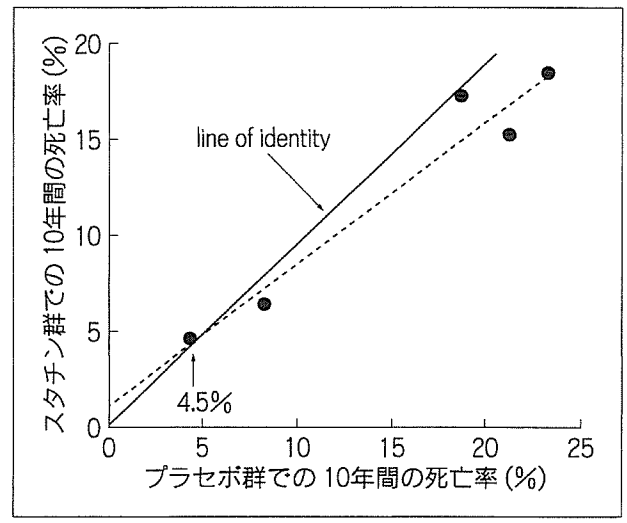

図4　スタタチン治療に関するL'Abbe plot
（文献5より引用）

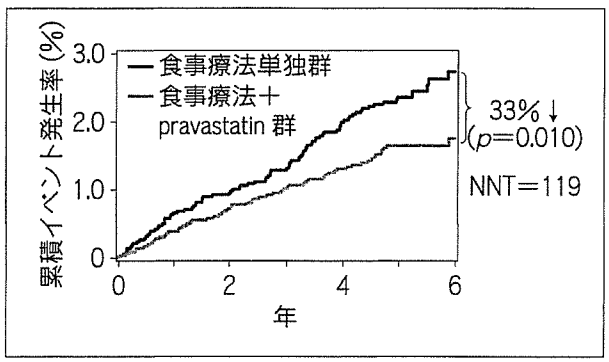

図5　一次評価項目：冠動脈疾患（CHD）

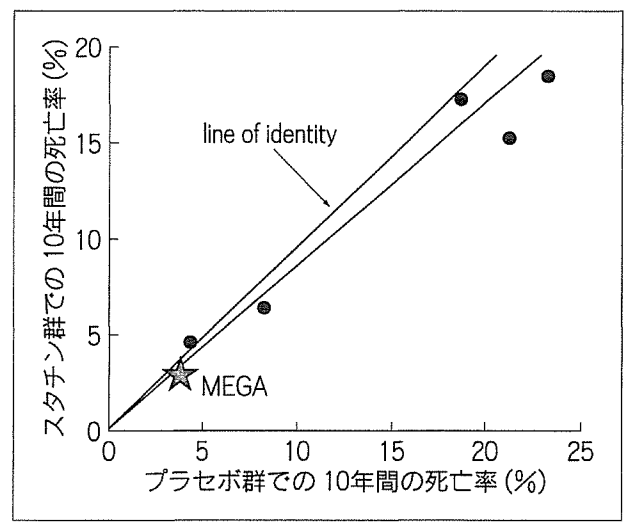

図6　MEGAによって修正されるスタチン治療に関するL'Abbe plot
MEGAでは10年間で予想される総死亡率が3.8%から2.7%へ低下

の臨床試験から得られた点も$y=x$（line of identity）より下に位置するときは，かなり確からしく「介入行為が有益である」と判断することができる．

全プロットに対する回帰直線が原点を通るとすれば，コントロール群でのイベント発生率が0%であるような対象に対して薬物を投与しても有害ではないことを示している．しかし，現実的には，薬物投与時に有害作用出現を0%とすることは困難であり，**図3**で示すようにy軸の正の部分に切片をもつ場合も出現する．複数の臨床試験結果から得られた近似線と$y=x$が交わるならば，そこが有益性と有害性の分岐点であり，同じ治療であっても患者のベースラインリスクによっては有益にも有害にもなることを意味する．交点のx値よりも患者のベースラインリスクが大きい場合には，介入行為の有益性が有害性にまさっており，ベースラインリスクが交点のx値よりも小さい場合には，介入行為が，むしろ有害であることを示している．

このように薬物による介入の利益は，患者のリスクに依存しており，スタチン治療も例外ではない．スタチン投与による冠動脈イベント抑制効果を検証した大規模臨床試験結果をL'Abbe Plotを

表4　スタチンの一次予防試験における脂質変化とCHDリスク低下率

試験名	LDL-C		CHD相対リスク低下(RRR)
	前値(mg/dl)	介入後(mg/dl)(%変化)	
WOSCOPS	192	142(−26)	−31
AFCAPS/TexCAPS	150	115(−25)	−37
ALLHAT-LLT	146	105(−28)	−9
ASCOT-LLA	133	87(−35)	−36
CARDS	118	71(−40)	−37
MEGA	157	128(−18)	−33

MEGA Study Group; AHA 2005

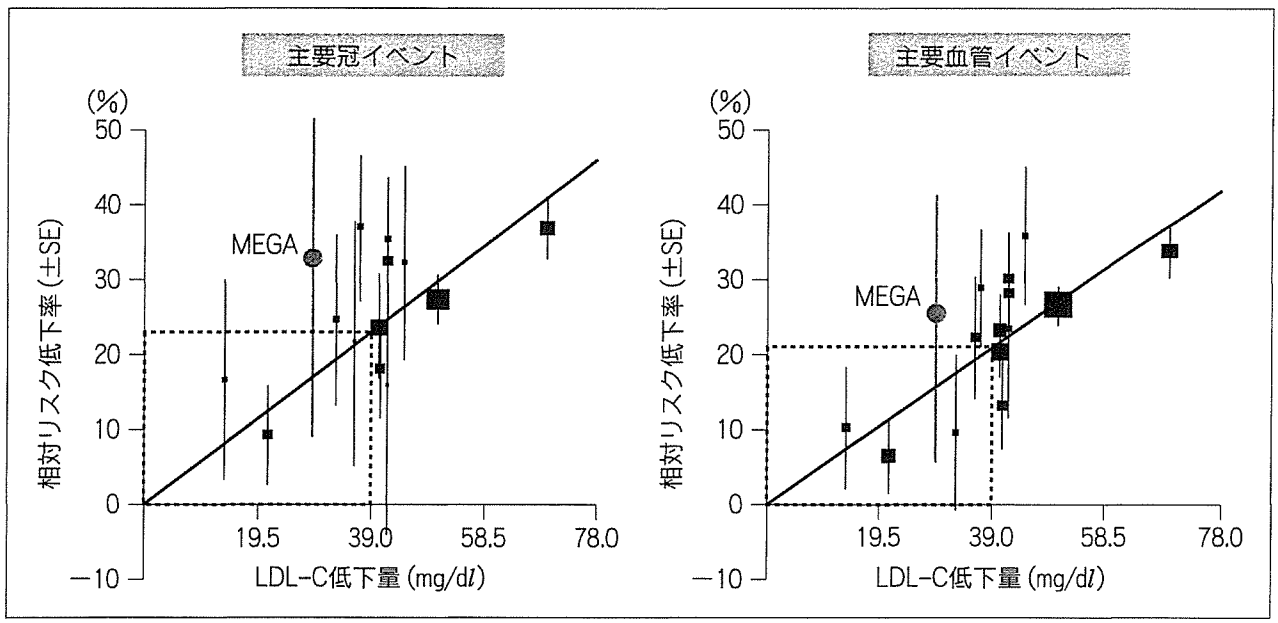

図7　スタチンの無作為化試験のメタアナリシスLDL-C低下量と主要イベント抑制作用

用い解析することで，どのようなイベント発生率をもつ対象に対してスタチン投与がなされるべきかを判断する重要な手がかりとなる．これまで海外で行われたスタチン治療の試験成績からは，10年間の死亡率が4.5%を超える群で有益であり(図4)，また発症率でいうならば13%を超える患者群でスタチン治療が死亡率改善に効果があることが報告されている[5]．このような報告に基づけば，WOSCOPSとJ-LITの結果からも明らかなように欧米に比べて冠動脈イベント発症率がはるかに低い日本において，スタチン治療の有益性がしばしば議論されてきたのも当然と考えられる．

海外と国内のエビデンスの比較

2005年の米国心臓病学会で発表されたMEGA Studyによって，わが国においてはじめて，軽度から中等度に相当する総コレステロール220〜270 mg/dlの高脂血症患者において，pravastatin治療が虚血性心疾患の一次予防に有効であることが示された[6,7]（図5）．ベースラインリスクが低い集団に対しても，スタチン治療が心血管イベント発症を抑制することを示した画期的な試験であり，今後の海外でのスタチン治療にも大きなインパクトを与えるであろう．

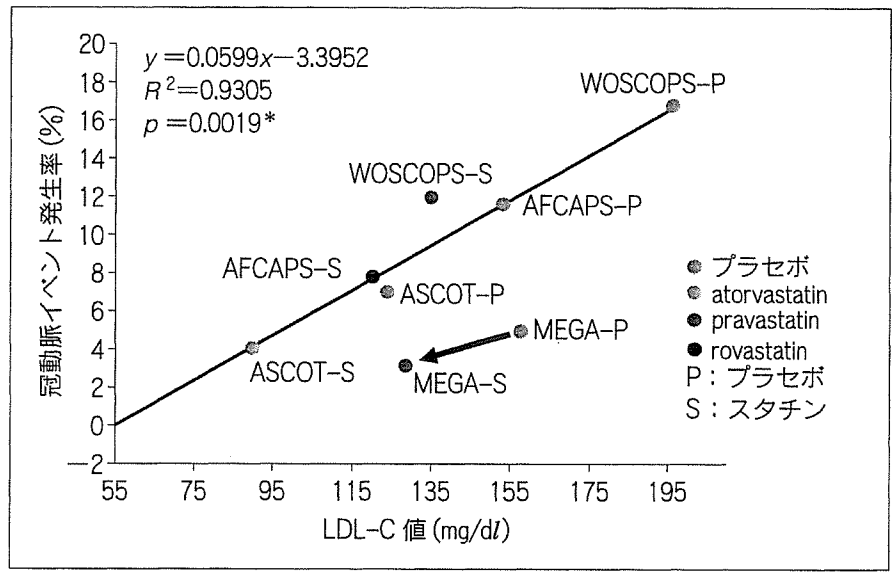

図8　スタチンによる冠動脈疾患一次予防
*unweighted regression lines を用いて解析
[O' Keefe JH et al: J Am Coll Cardiol 2004; **43**: 2142–2146より引用，改変]

単純にMEGA Studyの結果を欧米に外挿することは，海外での結果をそのまま国内に適用できないことと同様に慎重でなければならないが，仮にMEGA Studyの結果を上記のL'Abbe Plotに追加し再解析すると，スタチンの有益性と有害性の分岐点を大きく低リスク側に引き寄せ，かなりの低リスク集団までスタチンが死亡率改善に有益である可能性を示すこととなる（**図6**）．

さらにMEGA Studyでは，pravastatin群のLDL-C低下率は18%と，欧米で実施された大規模臨床試験結果と比較すると低いLDL-C低下率にもかかわらず，虚血性心疾患の相対リスク低下率は33%であり，欧米のLDL-C低下率25～40%の試験と同程度であった（**表4**）．これまでのスタチンを用いたランダム化試験のメタアナリシスから，LDL-C低下量と主要イベント抑制作用の関係を検討すると，LDL低下量が大きいほど相対リスク低下率が高くなる傾向が観察される（**図7**）．MEGA Studyの結果は，この図表中でもっとも左上に近くプロットされ，わずかなLDL-C低下で大きな相対リスク低下が得られたことを示している．またスタチンによる冠動脈疾患一次予防目的の臨床試験結果にMEGA Studyの結果を追加すると，海外での試験結果は同一線上にプロットされるのに対して，MEGA Studyの結果は離れた場所にプロットされる（**図8**）．この乖離は，海外と国内における虚血性心疾患発症率の相違のみならず，スタチン感受性の人種差，食生活の相違などさまざまな要因を想起させ興味深い．MEGA Studyは，これまでの試験の中でもっとも効率のよい試験結果であり，またLDL-C低下を超えた多面的作用がもっとも発揮された試験であったといえるのかもしれない．

おわりに

スタチン治療の有益性は，対象のイベント発生リスクによって大きく異なる．したがって日本人の高脂血症に対しては，日本における大規模臨床試験のエビデンスに基づきベースラインリスクを検討して治療にあたることが重要である．MEGA Studyは虚血性心疾患のリスクが低いといわれていた日本人において，はじめて欧米と同等のスタチン治療による虚血性イベント抑制効果を明らかにした．

一方，従来の欧米のエビデンスとは異なる知見や解決すべき疑問も提示されている．欧米の二次予防試験で示されるLDL-Cは低ければ低いほどよいとされるスタチン治療の効果が日本人においても適用可能か，あるいは，MEGA Studyで示

された効率的な虚血性心疾患発症の相対リスク低下が，真に日本人はスタチンの多面的作用の恩恵を享受しやすいことを示唆しているのかという疑問に答えるため，今後さらに，日本人を対象にしたエビデンスを構築していくことが重要と考えられる．

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Effects of Acute Administration of Caffeine on Vascular Function

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Caffeine is the most widely used pharmacologic substance in the world. It is found in common nonessential grocery items (e.g., coffee, tea, cocoa, and chocolate). The effects of caffeine on cardiovascular diseases, including hypertension, remain controversial, and there is little information on its direct effect on vascular function. The purpose of this study was to determine the effect of caffeine on endothelial function in humans. This study was a double-blind, randomized placebo and active drug study. Forearm blood flow (FBF) responses to acetylcholine (ACh), an endothelium-dependent vasodilator, and to sodium nitroprusside, an endothelium-independent vasodilator, were evaluated in healthy young men before and after the oral administration of caffeine 300 mg (n = 10) or placebo (n = 10). FBF was measured by using a strain-gauge plethysmograph. Caffeine significantly increased systolic and diastolic blood pressures by 6.0 ± 6.0 and 2.6 ± 3.1 mm Hg ($p < 0.05$), respectively, but did not alter heart rate or baseline FBF. Caffeine augmented the FBF responses to ACh from 21.2 ± 7.1 to 26.6 ± 8.1 ml/min/100 ml tissue ($p < 0.05$), whereas sodium nitroprusside–stimulated vasodilation was not altered by caffeine administration. The intra-arterial infusion of N^G-monomethyl-L-arginine, a nitric oxide synthase inhibitor, abolished the caffeine-induced augmentation of FBF response to ACh. In the placebo group, the ACh- and sodium nitroprusside–stimulated vasodilation was similar before and after the follow-up period. In conclusion, these findings suggest that the acute administration of caffeine augments endothelium-dependent vasodilation in healthy young men through an increase in nitric oxide production. © 2006 Elsevier Inc. All rights reserved. **(Am J Cardiol 2006;98:1538–1541)**

This study was designed to examine the effects of the acute administration of caffeine on systemic hemodynamics and endothelial function in humans by measuring forearm blood flow (FBF) responses to acetylcholine (ACh), an endothelium-dependent vasodilator, and to sodium nitroprusside (SNP), an endothelium-independent vasodilator.

Methods and Results

The subjects were 20 young healthy men recruited from healthy volunteers. All were nonhabitual caffeine consumers who did not consume caffeine every day. This study was a double-blind, randomized placebo and active drug study. The 20 subjects were randomly assigned to receive caffeine (caffeine group; n = 10, mean age 26.8 ± 5.2 years) or placebo (control group; n = 10, mean age 26.1 ± 3.8 years). The study protocol was approved by the ethics committee of the Hiroshima University Graduate School of Biomedical Sciences. Informed consent was obtained from all subjects before participation.

FBF was measured with the use of a mercury-filled Silastic strain-gauge plethysmograph (EC-5R, D.E. Hokanson, Inc., Bellevue, Washington), as previously described.[1,2] Three plethysmographic measurements were averaged to determine FBF at baseline and during the administration of each drug. FBF is expressed in milliliters per minute per 100 ml of forearm tissue volume. FBF was calculated by 2 independent observers blinded to the study protocol from the linear portions of plethysmographic recordings. The intraobserver coefficient of variation was 3.0%. We confirmed the reproducibility of FBF responses to ACh and

Table 1
Clinical characteristics of the control and caffeine groups

Variable	Control (n = 10)	Caffeine (n = 10)
Body mass index (kg/m^2)	22 ± 2	22 ± 1
Systolic blood pressure (mm Hg)	114 ± 7	117 ± 10
Diastolic blood pressure (mm Hg)	64 ± 8	61 ± 8
Heart rate (beats/min)	65 ± 7	61 ± 9
Total cholesterol (mmol/L)	4.12 ± 0.59	4.05 ± 0.65
Triglycerides (mmol/L)	1.27 ± 0.67	1.11 ± 0.55
Triglycerides (mg/dl)	112 ± 59	98 ± 49
Low-density lipoprotein cholesterol (mmol/L)	2.25 ± 0.54	2.12 ± 0.56
Low-density lipoprotein cholesterol (mg/dl)	87 ± 21	82 ± 22
Serum creatinine (μmol/L)	80 ± 9	79 ± 9
FBF (ml/min/100 ml tissue)	6.3 ± 2.8	6.1 ± 3.0

All results are expressed as mean ± SD.

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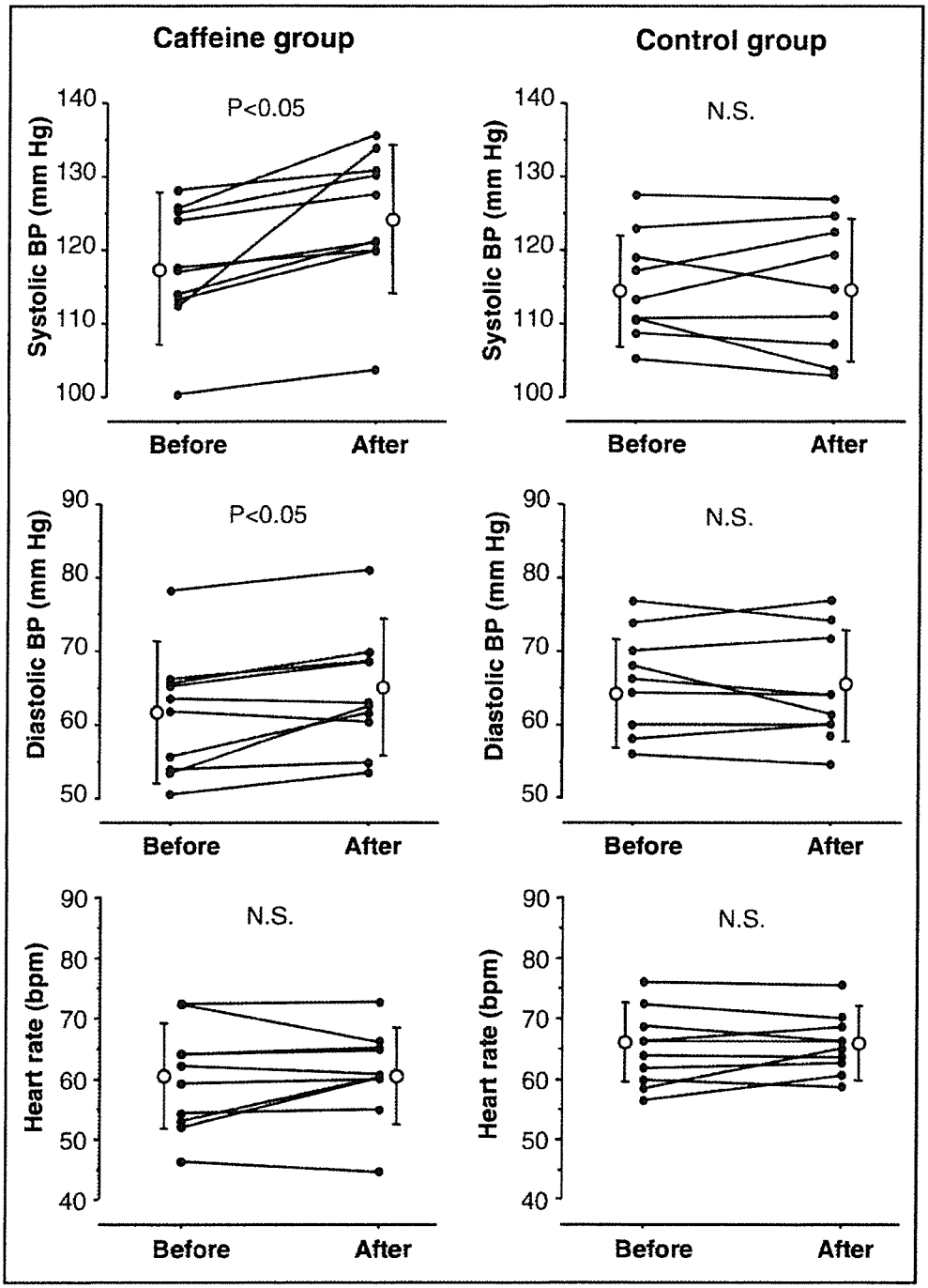

Figure 1. Effects of caffeine *(left)* and placebo *(right)* on systolic blood pressure (BP), diastolic BP, and heart rate.

SNP on 2 separate occasions in 10 healthy men (mean age 24 ± 4 years). The coefficients of variation were 6.2% and 4.6%, respectively.

All measurements were performed for subjects in the supine position in a temperature-controlled (22°C to 25°C), quiet, dark laboratory. All subjects abstained from caffeine, ethanol, and nicotine for ≥24 hours before the start of the study. After 30 minutes in the supine position, baseline FBF, heart rate, and arterial blood pressure were measured. Then the intra-arterial infusions of the endothelium-dependent vasodilator ACh (3.75, 7.5, and 15 μg/min) or the endothelium-independent vasodilator SNP (0.75, 1.5, and 3.0 μg/min) were performed randomly every 5 minutes, and FBF during the final 2 minutes of each infusion was measured.

After a 30-minute rest period, caffeine 300 mg or placebo was administered orally to each subject. Baseline FBF, heart rate, and arterial blood pressure were measured 1 hour after the oral administration of caffeine or placebo. The effects of ACh and SNP were determined again by the same

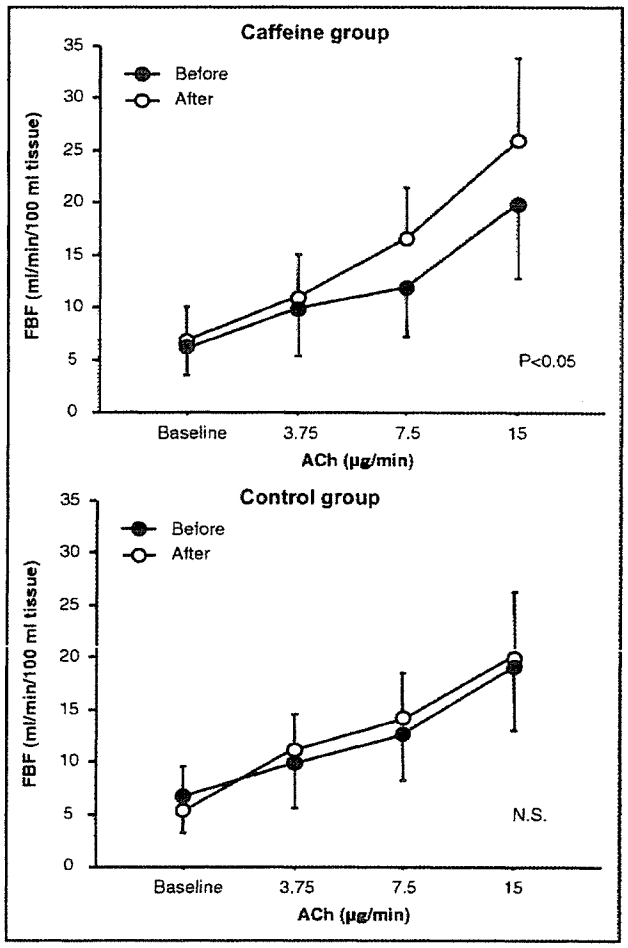

Figure 2. Effects of ACh on FBF before and after caffeine or placebo administration.

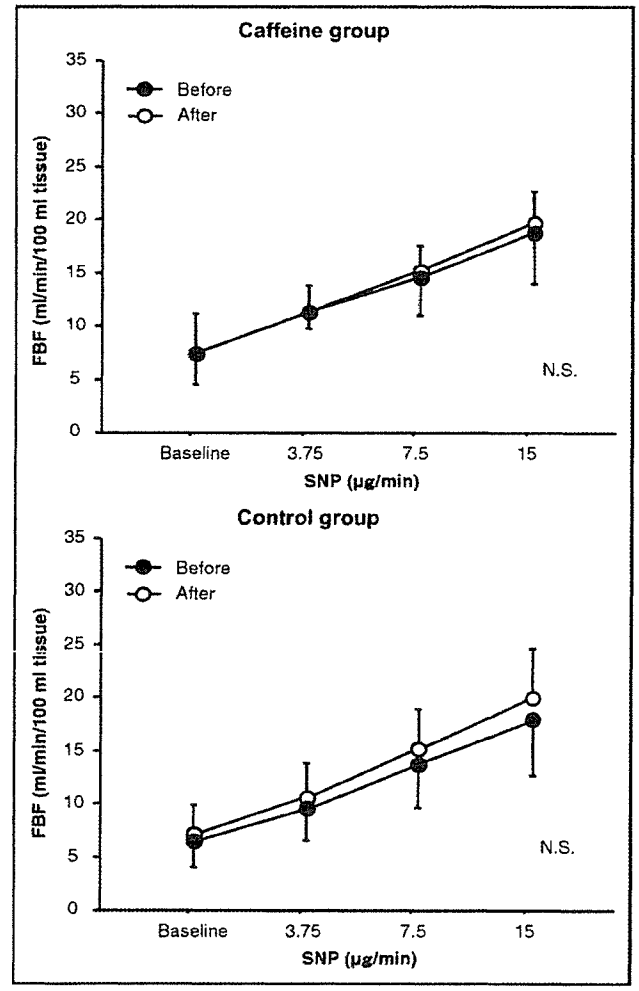

Figure 3. Effect of SNP on FBF before and after caffeine or placebo administration.

method as that used before caffeine and placebo administration.

After a 30-minute rest period, N^G-monomethyl-L-arginine (L-NMMA; Clinalfa Company, Läufelfiger, Switzerland), a nitric oxide synthase inhibitor, was infused intra-arterially at a dose of 8 μg/min for 5 minutes while baseline FBF and arterial blood pressure were recorded, and ACh (3.75, 7.5, and 15 μg/min) was administered.

The results are expressed as mean ± SD. Values of $p < 0.05$ were considered to indicate statistical significance. Baseline characteristics between 2 groups were compared using the Mann-Whitney U-statistic test. The effects of interventions on blood pressure, heart rate, and FBF were analyzed with the paired Student's *t* test. Comparisons of dose-response curves of parameters during the infusion of the drugs were analyzed with repeated-measures analysis of variance. The data were processed using the software package StatView V (SAS Institute Inc., Cary, North Carolina).

Baseline clinical characteristics in the caffeine group and control group are summarized in Table 1. There were no significant differences between the 2 groups in systolic blood pressure, diastolic blood pressure, heart rate, FBF, and other parameters.

Caffeine elevated systolic blood pressure from 117 ± 10 to 123 ± 9 mm Hg ($p < 0.05$) and elevated diastolic blood pressure from 61 ± 8 to 64 ± 8 mm Hg ($p < 0.05$) but did not alter heart rate (Figure 1). There were no significant differences in systolic and diastolic blood pressures or heart rate after placebo ingestion.

The intra-arterial infusion of ACh and SNP significantly increased FBF in a dose-dependent manner in the caffeine and control groups. FBF responses to ACh and SNP were similar in the 2 groups. Neither caffeine nor placebo altered baseline FBF. Caffeine significantly augmented FBF response to ACh ($p < 0.05$), whereas placebo did not alter FBF response to ACh (Figure 2). Neither caffeine nor placebo altered FBF response to SNP (Figure 3). No significant change was observed in arterial blood pressure or heart rate with the intra-arterial infusion of either ACh or SNP in any of the subjects.

The intra-arterial infusion of L-NMMA reduced baseline FBF and abolished the caffeine-induced augmentation of FBF response to ACh (Figure 4). No significant change was observed in arterial blood pressure or heart rate with the intra-arterial infusion of ACh in the presence of L-NMMA.

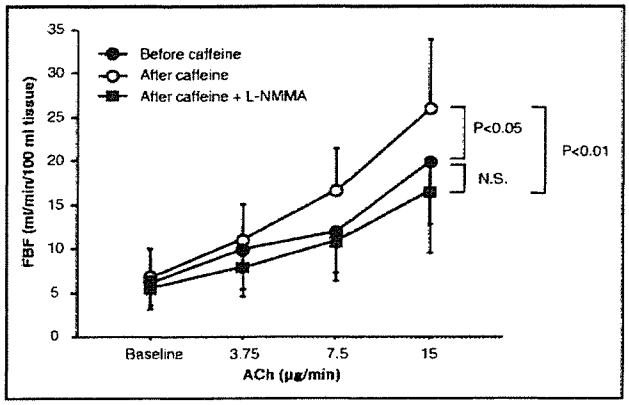

Figure 4. Effects of ACh on FBF in the presence of L-NMMA after caffeine administration.

Discussion

To our knowledge, this is the first study to demonstrate that the oral administration of caffeine increases FBF response to ACh, an endothelium-dependent vasodilator. In addition, L-NMMA completely abolished the caffeine-induced augmentation of FBF response to ACh. Caffeine elevated systolic and diastolic blood pressures but did not alter heart rate.

Some investigators have hypothesized that caffeine is a vasoconstrictive substance.[3-9] In the present study, systolic and diastolic blood pressures were elevated after caffeine ingestion, suggesting vasoconstrictive effects of caffeine.[5] Caffeine should be an antagonist of the adenosine receptor.[10,11] It is well known that adenosine induces vasodilation. Therefore, antagonization of the adenosine receptor could induce vasoconstriction. However, although oral caffeine ingestion did not change baseline FBF, FBF response to ACh was significantly increased in the caffeine group. Hatano et al[12] reported that caffeine promotes nitric oxide synthesis in the endothelium by the release of Ca^{2+} from the endoplasmic reticulum through activation of the ryanodine-sensitive Ca^{2+} channel and the suppression of cyclic guanosine monophosphate degradation in the isolated rat aorta, resulting in the caffeine-induced augmentation of endothelium-dependent vasodilatation. In the present study, L-NMMA, a nitric oxide synthase inhibitor, completely abolished the caffeine-induced augmentation of endothelium-dependent vasodilation. These findings suggest that caffeine augments endogenous nitric oxide production by agonist stimulation. A balance of the vasodilatory effect of caffeine as an endothelium-dependent vasodilator and the vasoconstrictive effect of caffeine as an adenosine receptor antagonist may regulate vascular function.

In the present study, caffeine ingestion elevated systolic and diastolic blood pressures in the brachial artery. Our results support those of previous studies showing that the acute administration of caffeine elevates peripheral blood pressure.[6-9] Karatzis et al[13] demonstrated the augmentation of central blood pressure after the acute administration of caffeine, but peripheral systolic blood pressure did not significantly change. It has been reported that various factors, such as hypertension, exercise stress, and age, influence blood pressure response to caffeine.[14] These observations suggest that the confounding factors should be kept fairly constant for the assessment of changes in blood pressure during caffeine administration.

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Roles of Rho-Associated Kinase and Oxidative Stress in the Pathogenesis of Aortic Stiffness

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Objectives The purpose of this study was to determine the relationship between Rho-associated kinase (ROCK) activity and aortic stiffness in humans.

Background Epidemiologic studies have shown that there is a relationship between aortic stiffness and cardiovascular complications. Recent evidence suggests that ROCK plays an important role in the process of atherosclerosis.

Methods We evaluated the forearm blood flow (FBF) response to sodium nitroprusside (SNP), a nitric oxide donor, acetylcholine (ACh), an endothelium-dependent vasodilator, and fasudil, a specific ROCK inhibitor, in 51 healthy male subjects (mean age 45.6 ± 3.0 years). The FBF was measured by using a strain-gauge plethysmography. Carotid-femoral pulse wave velocity (cf-PWV) was measured to assess the aortic stiffness using a pulse wave velocimeter.

Results Intra-arterial infusion of SNP alone, ACh alone, or fasudil alone and after co-infusion of N^G-monomethyl-L-arginine (L-NMMA), a nitric-oxide synthase inhibitor, significantly increased FBF in a dose-dependent manner ($p < 0.01$). Multivariate analysis showed that age and number of pack-years smoked were independent predictors of ROCK activity before or after co-infusion of L-NMMA ($p < 0.01$) and that age and ROCK activity before or after co-infusion of L-NMMA were independent predictors of cf-PWV ($p < 0.01$). The concentration of serum malondialdehyde-modified low-density lipoprotein, an index of oxidative stress, was significantly correlated with ROCK activity before and after co-infusion of L-NMMA and cf-PWV ($p < 0.01$).

Conclusions These findings suggest that aging and accumulating smoking habit, which might induce excessive oxidative stress, are involved in ROCK activity in the vasculature, leading to an increase in aortic stiffness in humans. (J Am Coll Cardiol 2007;49:698–705) © 2007 by the American College of Cardiology Foundation

The small guanosine triphosphatase (GTPase) Rho works as a switch and plays an important role in various cellular physiologic functions, including actomyosin-based cellular processes such as cell adhesion, migration, motility, cytokinesis, and contraction, all of which may be involved in the pathogenesis of atherosclerosis (1). There is growing evidence that Rho-associated kinase (ROCK) (also known as Rho-kinase), the immediate downstream target of the small GTP-binding protein Rho, contributes to endothelial dysfunction and vascular disease (2–6). Indeed, recent clinical evidence has demonstrated that ROCK is significantly activated in patients with coronary vasospasm (7), hypertension (8), and stable-effort angina (9) and even in current smoking subjects (10,11). ROCK, therefore, is becoming a new therapeutic target in cardiovascular disease. ROCK physiologically plays a key role in vasoconstriction. It activates myosin light chain kinase by phosphorylation of the myosin-binding subunit in myosin light chain phosphatase, leading to contraction of vascular smooth muscle cells (VSMC) (12,13). Thus, the vasoconstriction mediated by ROCK is dependent on Ca^{2+} sensitization but independent of Ca^{2+} concentration. Earlier studies have known that vascular dysfunction, including endothelial and VSMC dysfunction, is associated with cardiovascular risk factors such as aging, smoking habit, and oxidative stress (14,15), but little is known about the underlying correlations of

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ROCK with cardiovascular risk factors. In addition, there is no clinical evidence of a relationship between ROCK and oxidative stress, although recent studies have revealed a relationship between endothelial dysfunction and ROCK (3,4,11,16) and between endothelial dysfunction and oxidative stress (17,18). Several recent studies have provided evidence that reactive oxygen species activate the Rho/ROCK pathway (19,20). Indeed, we previously showed that there is a significant relationship between endothelial dysfunction and increased ROCK activity in young current smokers, which might be considered as a human model of excess oxidative stress compared with young nonsmokers (11). Taken together, these findings indicate that reactive oxygen species may play an important role in activation of ROCK in humans.

Epidemiologic and clinical studies have shown that impaired aortic stiffness, which can be assessed noninvasively by measurement of pulse wave velocity (PWV), is an independent marker to estimate subjects with cardiovascular disease (21,22). The PWV depends on arterial wall structure, mainly collagen and elastin, and arterial function (23). The elastic properties of the aorta and central arteries are the major determinants of systemic arterial impedance, and PWV measured along the aortic and aortoiliac pathway is the most clinically relevant.

To determine the roles of ROCK and oxidative stress in the pathogenesis of impaired aortic stiffness, we evaluated whether oxidative stress is related to ROCK activity and subsequently to aortic stiffness in humans.

Methods

Subjects. We studied 51 healthy male subjects (mean age 45.6 ± 3.0 years). Subjects with a history of hypertension, hypercholesterolemia, or diabetes mellitus were excluded. Normal blood pressure was defined as systolic blood pressure of <140 mm Hg and diastolic blood pressure of <85 mm Hg. The results of physical and routine laboratory examinations of the subjects were normal. None of the subjects were taking oral antioxidant vitamins or vasoactive drugs. Current smokers were defined as any who had smoked at least 1 pack-year. One pack-year was defined as 20 cigarettes per day for 1 year. All of the smokers (35.2 ± 5.2 pack-years) had a current smoking history of more than 5 years and abstained from smoking for at least 3 h before the forearm blood flow (FBF) measurements. We defined nonsmokers as those who had never smoked. The study protocol was approved by the Ethical Committee of Hiroshima University Graduate School of Biomedical Sciences. Informed consent for participation in the study was obtained from all subjects.

Procedures. Forearm vascular response to sodium nitroprusside (SNP) (Maluishi Pharmaceutical Co., Tokyo, Japan) alone, acetylcholine (ACh) (Daiichi Pharmaceutical Co., Tokyo, Japan) alone, and fasudil (Asahi Chemical Industries, Tokyo, Japan) alone and after co-infusion of N^G-monomethyl-L-arginine (L-NMMA) (Sigma Chemical Co., St. Louis, Missouri), were evaluated. The study began at 8:30 AM with the subjects in the fasting condition. A 23-gauge polyethylene catheter (Hakkow Co., Tokyo, Japan) was inserted into the left brachial artery for the infusion of each drug and for the recording of arterial pressure with an AP-641G pressure transducer (Nihon Kohden Co., Tokyo, Japan) under local anesthesia (1% lidocaine). Another catheter was inserted into the left deep antecubital vein to obtain blood samples.

Abbreviations and Acronyms

ACh = acetylcholine
cf-PWV = carotid-femoral pulse wave velocity
FBF = forearm blood flow
L-NMMA = N^G-monomethyl-L-arginine
MDA-LDL = malondialdehyde-modified low-density lipoprotein
PWV = pulse wave velocity
ROCK = Rho-associated kinase
SNP = sodium nitroprusside
VSMC = vascular smooth muscle cells

After 30 min in the supine position, we measured basal FBF and arterial blood pressure. Then forearm vascular response to SNP, a direct vasodilator of VSMCs, ACh, an endothelium-dependent vasodilator, and fasudil, a specific ROCK inhibitor, on forearm hemodynamics were measured. The SNP (0.75, 1.5, or 3.0 μg/min), ACh (3.75 or 7.5 μg/min), and fasudil (3, 10, or 30 μg/min) were infused intra-arterially for 5 min at each dose. The FBF was measured during the last 2 min of the infusion. The infusions of SNP, ACh, and fasudil were carried out in a randomized fashion. Each study proceeded after the FBF returned to baseline.

After a 30-min rest period, L-NMMA, an inhibitor of nitric oxide synthase, was infused intra-arterially at a dose of 8 μmol/min for 5 min, and fasudil was administered 5 min after the initiation of L-NMMA.

To evaluate the relationship among ROCK activity, oxidative stress, and aortic stiffness, we studied 35 healthy male subjects (mean age 45.0 ± 3.6 years), whose prespecified entry criteria are identical to that described in the preceding. Number of pack-years smoked in smokers was 29.0 ± 6.6 pack-years. The PWV was measured in the supine position after 15 minutes of bed rest, and then forearm vasodilative responses to SNP alone, ACh alone, and fasudil alone and after co-infusion of L-NMMA, were evaluated in a manner identical to that described in the preceding text.

Measurement of FBF. The FBF was measured with a mercury-filled Silastic strain-gauge plethysmography (EC-5R, D. E. Hokanson, Issaquah, Washington) as previously described (11,18).

Measurement of PWV. Aortic compliance was assessed noninvasively on the basis of Doppler ultrasound measurements of PWV along the descending thoracoabdominal aorta as previously published and validated (24). Briefly,

carotid-femoral PWV (cf-PWV), an index of arterial stiffness, was determined by 2 pressure sensors, placed on the right femoral and left carotid arteries to record each pulse wave simultaneously, and the time lag (t) between the notches of the 2 waves, using a pulse wave velocimeter (Form PWV/ABI, model BP-203RPE, Colin Co., Aichi, Japan). The distance (D) between the 2 recording sensors was calculated automatically from the value of individual height. The PWV value was calculated as PWV = D/t. The PWV was measured for 5 consecutive pulses, and averages were used for analysis. The observer was blind to the form of the examination.

Analytical methods. Routine chemical methods were used to determine serum concentrations of total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, glucose, and insulin. The serum concentration of malondialdehyde-modified low-density lipoprotein (MDA-LDL) was assayed by ELISA (antiMDA-LDL antibody, SRL Co., Atsugi, Japan). Fasting concentrations of insulin and glucose were used to determine homeostatic model of assessment (HOMA) parameters of insulin resistance using a program based on the HOMA algorithm (insulin resistance = insulin/22.5$e^{-\ln \text{ glucose}}$), as previously described (25).

Statistical analysis. Results are presented as mean ± SEM. Values of $p < 0.05$ were considered to indicate statistical significance. Comparisons of time-course curves of variables during the infusion of SNP alone, ACh alone, and fasudil alone and after co-infusion of L-NMMA, were analyzed by analysis of variance (ANOVA) for repeated measures with Bonferroni correction to baseline. The ROCK activity was expressed as the percentage in the ratio of fasudil-stimulated maximal FBF relative to the immediately preceding basal FBF. Spearman rank correlation was used to compare ROCK activity before or after co-infusion of L-NMMA with age, body mass index (BMI), systolic blood pressure, diastolic blood pressure, heart rate, serum concentration of total cholesterol, HDL cholesterol, triglycerides, mean fasting glucose, insulin, MDA-LDL, and number of pack-years smoked. The analysis was also used to compare the ratio of SNP-stimulated maximal FBF to basal FBF or ACh-stimulated maximal FBF to basal FBF (endothelial function) with the variables and to compare the PWV with the variables in which endothelial function and ROCK activity before or after L-NMMA were added. Multivariate analysis using multiple stepwise regression was performed to determine the significant correlation of ROCK activity before or after co-infusion of L-NMMA and of PWV with variables which showed a p value of <0.1 in Spearman correlation analysis. Multivariate analysis was performed with the Statistical Analysis System program package (SAS Institute, Cary, North Carolina). The data were analyzed using the software package StatView V (SAS Institute) and Super ANOVA (Abacus Concepts, Berkley, California).

Table 1 Baseline Clinical Characteristics in the Subjects

Variable	Subjects (n = 51)
Age (yrs)	45.6 ± 3.0
Body mass index (kg/m^2)	23.5 ± 0.4
Systolic blood pressure (mm Hg)	123.6 ± 1.4
Diastolic blood pressure (mm Hg)	65.5 ± 1.4
Heart rate (beats/min)	62.5 ± 1.1
Total cholesterol (mmol/l)	4.70 ± 0.10
HDL cholesterol (mmol/l)	1.33 ± 0.06
Triglyceride (mmol/l)	1.03 ± 0.03
Mean fasting glucose (mmol/l)	5.1 ± 0.1
Serum insulin (pmol/l)	73.9 ± 16.6
Insulin resistance (HOMA index)	3.17 ± 0.81
Current smoker	27
Number of pack-yrs smoked	35.2 ± 5.2
Basal FBF (ml/min/100 ml tissue)	7.2 ± 0.4

All results are presented as mean ± SEM.
FBF = forearm blood flow; HDL = high-density lipoprotein; HOMA = homeostatic model assessment.

Results

Clinical characteristics. The baseline clinical characteristics of the subjects are summarized in Table 1.

Effects of FBF responses to SNP, ACh, and fasudil. Intra-arterial infusion of SNP significantly increased FBF in a dose-dependent manner by ANOVA for repeated measurements (6.1 ± 0.3 ml/min to 31.1 ± 1.4 ml/min per 100 ml tissue; $p < 0.01$) (Fig. 1). Intra-arterial infusion of ACh also significantly increased FBF in a dose-dependent manner by ANOVA for repeated measurements (7.7 ± 0.6 ml/min to 16.6 ± 1.5 ml/min per 100 ml tissue; $p < 0.01$) (Fig. 2). Intra-arterial infusion of fasudil significantly increased FBF in a dose-dependent manner alone and after co-infusion of L-NMMA by ANOVA for repeated measurements (7.0 ± 0.4 ml/min to 12.3 ± 0.6 ml/min per 100 ml tissue, 5.2 ± 0.4 ml/min vs. 10.3 ± 0.7 ml/min per 100 ml tissue, respectively; $p < 0.01$) (Fig. 3). No significant change was observed in arterial blood pressure or heart rate during intra-arterial infusion of any drugs.

Correlations with ROCK activity before and after L-NMMA. ROCK was significantly correlated with age ($r = 0.64$; $p < 0.001$), systolic blood pressure ($r = 0.44$; $p < 0.01$), serum concentration of total cholesterol ($r = 0.41$; $p < 0.01$), and number of pack-years smoked ($r = 0.52$; $p < 0.001$). The ROCK activity after co-infusion of L-NMMA was significantly correlated with age ($r = 0.64$; $p < 0.01$), systolic blood pressure ($r = 0.40$; $p < 0.01$), serum concentration of total cholesterol ($r = 0.47$; $p < 0.01$), and number of pack-years smoked ($r = 0.49$; $p < 0.01$). The ROCK activity before or after co-infusion of L-NMMA was not correlated with other parameters. The correlations between ROCK activity before or after co-infusion of L-NMMA and variables are summarized in Table 2. Stepwise multiple regression analysis was carried out to identify the independent predictors of ROCK activity before or after co-infusion

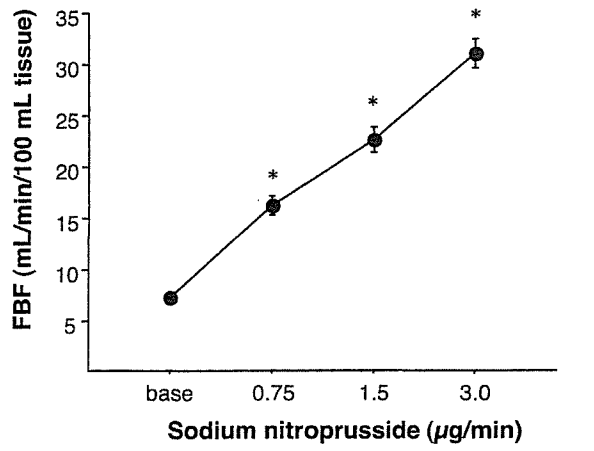

Figure 1 FBF Response to SNP

Results are presented as mean ± SEM. The probability value refers to a comparison of time course curves by analysis of variance for repeated measurements. *p < 0.01 versus basal forearm blood flow (FBF) assessed by Bonferroni correction. SNP = sodium nitroprusside.

of L-NMMA. For multiple regression analysis, variables showing a p value of <0.1 in Spearman correlation analysis were selected; age, systolic blood pressure, serum concentration of total cholesterol, and number of pack-years smoked were entered as candidates for independent variables. Stepwise multiple regression analysis revealed that age (standardized r = 0.36) and number of pack-years smoked (standardized r = 0.39) were independent predictors of

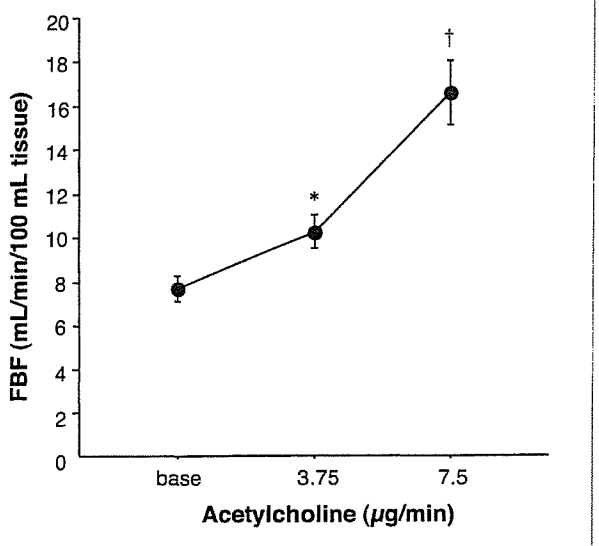

Figure 2 FBF Response to ACh

Results are presented as mean ± SEM. The probability value refers to a comparison of time course curves by analysis of variance for repeated measurements. *p < 0.05; †p < 0.01 versus basal forearm blood flow (FBF) assessed by Bonferroni correction. ACh = acetylcholine.

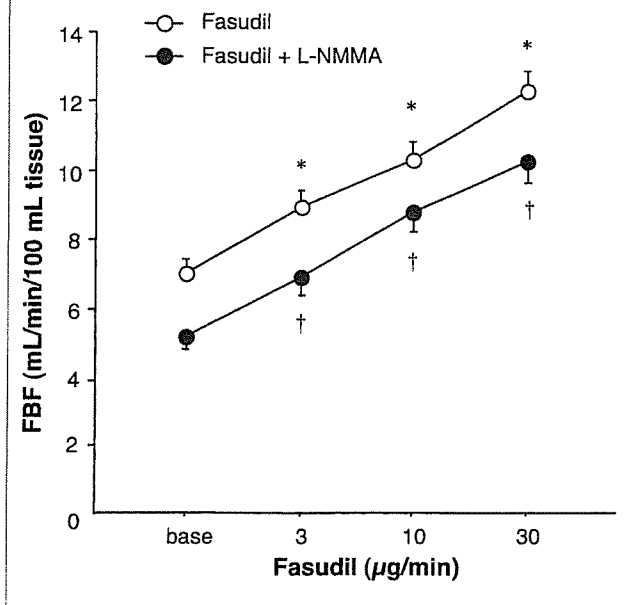

Figure 3 FBF Response to Fasudil Alone and After Co-Infusion of L-NMMA

Results are presented as mean ± SEM. The probability value refers to a comparison of time course curves by analysis of variance for repeated measurements in each group. *p < 0.01 versus basal forearm blood flow (FBF) of fasudil alone; †p < 0.01 versus basal FBF after co-infusion of N^G-monomethyl-L-arginine (L-NMMA) assessed by Bonferroni correction.

ROCK activity (multiple $R^2 = 0.43$; $p < 0.01$) and that age (standardized r = 0.34) and number of pack-years smoked (standardized r = 0.35) were independent predictors of ROCK activity after co-infusion of L-NMMA (multiple $R^2 = 0.37$; $p < 0.01$). The serum concentration of MDA-LDL was significantly correlated with ROCK activity before and after co-infusion of L-NMMA (r = 0.57 and r = 0.51, respectively; $p < 0.01$).

Correlations with maximal FBF response to SNP. There was no variable that was significantly correlated with the ratio of SNP-stimulated maximal FBF to basal FBF (Table 3). The serum concentration of MDA-LDL was also not significantly correlated with FBF response to SNP (r = −0.04; p = 0.81).

Correlations with endothelial function. Endothelial function was significant correlated with age (r = −0.55; $p < 0.01$), serum concentration of total cholesterol (r = −0.67; $p < 0.01$), and number of pack-years smoked (r = −0.59; $p < 0.01$) (Table 4). The serum concentration of MDA-LDL was also significantly correlated with endothelial function (r = −0.64; $p < 0.01$).

Correlations with cf-PWV. The cf-PWV was significantly correlated with age (r = 0.88, $p < 0.01$), systolic blood pressure (r = 0.42; $p < 0.05$), serum concentration of total cholesterol (r = 0.47; $p < 0.01$), number of pack-years smoked (r = 0.38; $p < 0.05$), endothelial function (r = −0.54; $p < 0.01$), ROCK activity (r = 0.67; $p < 0.01$), and ROCK activity after co-infusion of L-NMMA (r = 0.75;

Table 2 Correlations Between ROCK Activity Before and After L-NMMA and Variables

Variable	ROCK Activity Before L-NMMA		ROCK Activity After L-NMMA	
	Coefficient	p Value	Coefficient	p Value
Age (yrs)	0.636	<0.001	0.635	<0.001
Body mass index (kg/m^2)	−0.019	0.892	−0.007	0.961
Systolic blood pressure (mm Hg)	0.437	0.002	0.396	0.007
Diastolic blood pressure (mm Hg)	−0.093	0.516	−0.134	0.358
Heart rate (beats/min)	−0.089	0.533	−0.131	0.368
Total cholesterol (mmol/l)	0.414	0.004	0.471	0.001
HDL cholesterol (mmol/l)	−0.157	0.277	−0.156	0.290
Triglyceride (mmol/l)	0.167	0.248	0.089	0.547
Mean fasting glucose (mmol/l)	0.097	0.555	−0.048	0.778
Serum insulin (pmol/l)	0.066	0.677	0.087	0.590
Insulin resistance (HOMA index)	0.033	0.852	0.026	0.888
Number of pack-yrs smoked	0.515	<0.001	0.487	<0.001

L-NMMA = N^G-monomethyl-L-arginine; ROCK = Rho-associated kinase; other abbreviations as in Table 1.

$p < 0.01$). The ratio of SNP-stimulated maximal FBF to basal FBF was not significantly correlated with cf-PWV. The cf-PWV was not correlated with other parameters. The correlations between cf-PWV and variables are summarized in Table 5. For multiple regression analysis, variables showing a p value of <0.1 in Spearman correlation analysis were selected; age, systolic blood pressure, serum concentration of total cholesterol, number of pack-years smoked, endothelial function, and ROCK activity before or after L-NMMA, were entered as candidates for independent variables. Stepwise multiple regression analysis revealed that age (standardized r = 0.71) and ROCK activity (standardized r = 0.27) were independent predictors of cf-PWV (multiple $R^2 = 0.79$; $p < 0.01$) and that age (standardized r = 0.51) and ROCK activity after co-infusion of L-NMMA (standardized r = 0.54) were independent predictors of cf-PWV (multiple $R^2 = 0.91$; $p < 0.01$). In addition, the serum concentration of MDA-LDL was significantly correlated with cf-PWV ($r = 0.57$; $p < 0.01$).

Discussion

In the present study, there were significant relationships between ROCK activity and age, systolic blood pressure, serum concentration of total cholesterol, and number of pack-years smoked in healthy male subjects. In multivariate analysis, age and number of pack-years smoked were independent predictors of ROCK activity among the candidates that were correlated with ROCK activity. In addition, the concentration of serum MDA-LDL, one of the established markers of oxidative stress, was correlated with ROCK activity. To the best of our knowledge, this is the first study to provide clinical evidence revealing significant involvement of ROCK activity with age, accumulating current smoking habit, and oxidative stress in humans.

Recently, increasing evidence has indicated that ROCK is significantly associated with the regulation of not only endothelial nitric oxide synthase (eNOS) expression but also eNOS phosphorylation, both of which are important mechanisms for regulating endothelial function and subsequent cardiovascular injury (2–4,16). We have also demonstrated a significant relationship between endothelial dysfunction

Table 3 Correlations Between the Ratio of SNP-Stimulated Maximal FBF to Basal FBF and Variables

Variables	Coefficient	p Value
Age (yrs)	−0.127	0.372
Body mass index (kg/m^2)	−0.049	0.730
Systolic blood pressure (mm Hg)	0.107	0.453
Diastolic blood pressure (mm Hg)	0.040	0.780
Heart rate (beats/min)	−0.086	0.546
Total cholesterol (mmol/l)	−0.120	0.407
HDL cholesterol (mmol/l)	0.005	0.972
Triglyceride (mmol/l)	0.147	0.308
Mean fasting glucose (mmol/l)	0.049	0.768
Serum insulin (pmol/l)	−0.045	0.776
Insulin resistance (HOMA index)	−0.044	0.801
Number of pack-yrs smoked	0.021	0.886

SNP = sodium nitroprusside; other abbreviations as in Table 1.

Table 4 Correlations Between Endothelial Function and Variables

Variables	Coefficient	p Value
Age (yrs)	−0.548	0.003
Body mass index (kg/m^2)	−0.020	0.915
Systolic blood pressure (mm Hg)	−0.313	0.092
Diastolic blood pressure (mm Hg)	0.140	0.451
Heart rate (beats/min)	0.218	0.241
Total cholesterol (mmol/l)	−0.671	<0.001
HDL cholesterol (mmol/l)	0.071	0.709
Triglyceride (mmol/l)	−0.074	0.694
Mean fasting glucose (mmol/l)	0.281	0.187
Serum insulin (pmol/l)	0.179	0.353
Insulin resistance (HOMA index)	0.346	0.113
Number of pack-yrs smoked	−0.588	0.002

Abbreviations as in Table 1.

Table 5 Correlations Between Pulse Wave Velocity and Variables

Variables	Coefficient	p Value
Age (yrs)	0.882	<0.001
Body mass index (kg/m^2)	0.025	0.884
Systolic blood pressure (mm Hg)	0.418	0.015
Diastolic blood pressure (mm Hg)	0.211	0.218
Heart rate (beats/min)	−0.141	0.411
Total cholesterol (mmol/l)	0.472	0.006
HDL cholesterol (mmol/l)	−0.085	0.620
Triglyceride (mmol/l)	0.133	0.437
Mean fasting glucose (mmol/l)	−0.069	0.695
Serum insulin (pmol/l)	−0.060	0.729
Insulin resistance (HOMA index)	−0.145	0.421
Number of pack-yrs smoked	0.377	0.028
The ratio of SNP-stimulated maximal FBF to basal FBF (%)	−0.155	0.373
Endothelial function (%)	−0.543	0.009
ROCK activity (%)	0.670	<0.001
ROCK activity after coinfusion of L-NMMA (%)	0.746	<0.001

Abbreviations as in Tables 1, 2, and 3.

and increased ROCK activity in current smokers (11). The results of the present study show that aging and cigarette smoking are involved in an increase in ROCK activity, which might be partly explained by the significant correlation between ROCK and endothelial function. Our results are supported by several recent studies showing that aging is significantly related to activation of the Rho/ROCK pathway (26,27). Those findings suggest that activation of ROCK is involved in several aspects of the atherosclerotic process, including endothelial dysfunction.

Fasudil, which competes with adenosine 5'-triphosphate (ATP) for binding to ATP-dependent kinase domains, has recently been shown to be a potent and specific inhibitor of ROCK (5,6,28). ROCK activity in humans has been investigated in several previous studies using fasudil (8,29). Masumoto et al. (7). demonstrated that ROCK activity, evaluated by the vasodilative response to fasudil, is increased at the segment of coronary vasospasm in patients with vasospastic angina. In addition, we evaluated ROCK activity after co-infusion of L-NMMA to assess ROCK activity in the forearm vasculature. Although it is not clear whether ROCK activity before or after co-infusion of L-NMMA is appropriate for assessing ROCK activity, ROCK activity after co-infusion of L-NMMA may be appropriate for precise assessment of ROCK activity because it may avoid the contribution of endogenous nitric oxide to ROCK in VSMC. Interestingly, we could not find a significant correlation of forearm vasodilatory response to SNP with the variables in this study, which is in accordance with previous evidence (8,18). This discrepancy between the vasodilative effects of fasudil and SNP may be due to the different pharmacologic mechanisms of the drugs, dependency on Ca^{2+} sensitivity, or Ca^{2+} concentration in VSMC.

One of the novel findings in the present study is that ROCK activity, but not endothelial function, is one of the independent predictors of increased PWV, indicating that ROCK is significantly involved in the pathogenesis of aortic stiffness. Aortic stiffness has been shown to be closely associated with cardiovascular risks (22,24,30) and to be a significant predictor of cardiovascular morbidity and mortality in subjects with a smoking habit, hypertension, end-stage renal disease, and aging (21,31,32). Of great interest are the results of a recent study by Willum-Hansen et al. (33) showing that PWV predicted a composite of cardiovascular outcomes over a median follow-up period of 9.4 years beyond 24-h mean arterial pressure and other traditional risk factors such as gender, age, BMI, current smoking habit, and alcohol intake in middle-aged and elderly individuals, results that are significant from the point of view of clinical benefit. According to previous studies, ROCK plays an important role in atherosclerotic processes, especially in VSMC (5,6,12,13,28), indicating that ROCK modulates VSMC function via several kinds of mechanisms, including regulation of Ca^{2+} sensitivity, which may explain the discrepancy in the results of the present study showing that ROCK activity but not endothelial function is an independent predictor of PWV.

Oxidative stress is known to be crucial for the development of cardiovascular disease and subsequent mortality (15,34). In the present study, we measured the concentration of serum MDA-LDL to evaluate the contribution of oxidative stress to ROCK activity and aortic stiffness. Thus, the significant correlations of ROCK activity with age and number of pack-years smoked may be evoked partly through excess oxidative stress. Indeed, several investigators have demonstrated a possible correlation of ROCK with oxidative stress in in vitro and in vivo studies (20). Consequently, it is feasible that oxidative stress is significantly correlated with ROCK activity and further aortic stiffness in the present study. Collectively, these findings support our hypothesis that activation of ROCK in VSMC leads to impaired aortic stiffness, although we could not determine from the results of the present study whether increased ROCK activity causes oxidative stress or whether oxidative stress caused by various variables such as aging and accumulating smoking habit leads to increased ROCK activity. Furthermore, endothelial dysfunction, which is significantly related not only to excess oxidative stress but also to activated ROCK, may also be an important mechanism of impairment of aortic stiffness. Although the precise mechanism remains to be determined, ROCK plays a critical role in the modulation of aortic stiffness through a pathway in which oxidative stress is involved.

Study limitations. Although, we obtained the striking finding that aortic stiffness significantly correlates with ROCK and oxidative stress, several limitations remain in the present study. It has been shown that creatinine clearance is negatively associated with PWV in subjects with moderate reduction of creatinine clearance (35). Although serum concentrations of creatinine in all subjects in this study were normal (<106 μmol/l), additional data on the

correlation of creatinine clearance with ROCK or PWV might make the results more plausible. In addition, recent studies have revealed significant relationships between aortic stiffness and several candidates such as serum high-sensitivity C-reactive protein (hs-CRP) and criteria for the diagnosis of the metabolic syndrome (36–38). Indeed, it has been shown that CRP activates ROCK, leading to plasminogen activator inhibitor-1 expression and atherothrombogenesis in vitro. Those findings suggest that serum level of hs-CRP may correlate with ROCK activity and may cause the elevation of PWV in humans (39). In the present study, waist circumference was not measured. The BMI was measured in all subjects instead of waist circumference, and BMI was <30 kg/m^2 in all subjects. Accordingly, our results might not have been greatly influenced by the lack of measurement of waist circumference. It is thought that many disorders of biologic and physiologic factors are related to impaired aortic stiffness. Therefore, further investigations are required to clarify the precise mechanism underlying impairment of aortic stiffness.

Conclusions. We have demonstrated that ROCK activity in the forearm vasculature is significantly associated with age and number of pack-years smoked and that increased cf-PWV is significantly related to age and ROCK activity. In addition, excess oxidative stress is significantly correlated with increased ROCK activity and PWV. These findings suggest that excessive oxidative stress might be involved in increased ROCK activity in the vasculature, leading to impaired aortic stiffness, and that not only oxidative stress but also ROCK might be vital therapeutic targets for cardiovascular protection.

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