原　著

高齢虚血性心疾患患者の退院後の身体活動強度は下肢筋力だけでなくバランス機能の影響を受けている

Not only leg strength but also balance function influences moderate-intensity physical activity after hospital discharge in elderly patients with ischemic heart disease

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抄　録

【目　的】虚血性心疾患（IHD）患者の退院後における中強度以上の身体活動時間を調査し，運動機能との関係を壮年者と高齢者で比較検討することを目的とした．

【方　法】入院期心臓リハビリテーションを処方されたIHD患者を壮年群と高齢群の2群に分け，各群の退院時の運動機能〔膝伸展筋力，片脚立位時間（OLST），Functional Reach（FR），および姿勢安定度評価指標（IPS）〕を調査した．また，各群の退院後2週間の中強度以上の身体活動時間を調査し，一日あたりの平均値を算出した．

【結　果】壮年群では中強度以上の身体活動時間は膝伸展筋力とのみ有意な正の相関を示した（$p < 0.05$）．一方，高齢群では中強度以上の身体活動時間は膝伸展筋力，OLST，FR，およびIPSと有意な正の相関を認めた（それぞれ，$p < 0.05$）．

【結　論】高齢IHD患者の退院後の中強度以上の身体活動時間は壮年心疾患患者とは異なり，下肢筋力に加えてバランス機能が影響していた．

〔心臓リハビリテーション（JJCR）17（1）：98–102，2012〕

Key words：虚血性心疾患，高齢者，身体活動，運動強度，バランス機能

1．はじめに

虚血性心疾患（IHD）患者の退院後の身体活動量を高く維持することは，その後の再発率の減少やQuality Of Lifeの改善に大きく寄与する[1]ことが知られている．とくに最近では，身体活動量の構成要素である活動強度が注目されており，低強度〔3 metabolic equivalents（METs）未満〕に比べて，中強度以上（3 METs以上）の活動時間のほうがその後の心血管イベント率や生存率と密接に関連している[2]ことが報告されている．このため，IHD患者の退院後の身体活動量を高める際には，単に活動時間だけでなく，活動強度を中強度以上に保つ必要があるが，その具体的な運動指導方法について検討した報告は極めて少ないのが現状である．さらに，IHD患者は高齢者の割合が高く，高齢IHD患者の運動機能は壮年IHD患者と比べて低下の度合いが大きいことから[3]，高齢者と壮年者に分けて退院後の運動指導方法を検討する必要がある．

そこで本研究は，IHD患者の退院後における中強度以上の身体活動時間を調査し，下肢筋力とバランス機能を含めた運動機能との関係を壮年者と高齢者との間で比較検討することを目的とした．

2. 対 象

2007年1月から2010年5月までの間に北里大学病院心臓血管センターに入院し，心臓リハビリテーションを処方されたIHD患者連続607例のうち，後述する除外基準に該当する者を除き，かつ退院時の運動機能および退院後の身体活動を測定できた65例を対象とした．

除外基準は整形外科疾患を有する者，中枢神経系疾患により運動麻痺を呈する者，認知症を有する者，維持血液透析を行っている者，運動機能測定時に歩行が自立していない者および歩行時に歩行補助具を使用している者とした．なお，患者に対して本研究の意義と運動機能の測定に関する注意事項，さらに本研究に対する同意の可否が治療方針や治療内容に影響しないことを十分に説明し，同意を得た後に本研究を実施した．

3. 方 法

a) 測定項目

1) 臨床的背景因子

年齢，性別，診断名，在院日数，左室駆出率および脳性ナトリウム利尿ペプチド（BNP）を調査した．

2) 身体活動

身体活動の指標として，一日の平均歩数，中強度以上（3 METs以上）の身体活動時間を調査した．測定機器は加速度計付き歩数計（ライフコーダPLUS，スズケン）を使用した．ライフコーダには上下方向の加速度センサーが搭載されており，その加速度をもとにして身体活動強度が算出される．また，ライフコーダが示す身体活動強度は4以上が3 METs以上の強度に相当する[4]．そこで本研究では，ライフコーダで記録できた身体活動時間のうち，4以上の身体活動時間を中強度以上の身体活動時間とした．測定期間は16日間とし，配布日と回収日を除いた14日間の起床から就寝までとした．なお，各身体活動量の指標は1日の平均値を算出し，解析値とした．

3) 運動機能

i) 下肢筋力

下肢筋力の指標として，等尺性膝伸展筋力（膝伸展筋力）を採用した．測定機器はHand-held Dynamometer（μTasMT-1, ANIMA）を使用した．測定肢位は股関節と膝関節が90°屈曲位の椅子坐位とし，圧力センサーが腓骨外果より2横指上にくるようにNKテーブルに固定した．測定は，1回5秒間で左右2回ずつ行い，左右の平均値を体重で除した値（% BW）を解析値とした．

ii) バランス機能

静的バランス機能の指標として，片脚立位時間（OLST），動的バランス機能の指標として，Functional Reach（FR）および姿勢安定度評価指標（IPS）を採用した．

OLSTは，測定の開始肢位を開眼にて両上肢を腰にあてた状態とした．測定手順は，左右どちらか任意の足で測定した．中止基準は，(a)軸足が移動する，(b)挙上した下肢が軸足に触れる，(c)軸足の足底以外の部分が床や壁に触れる，(d)両手または片手が腰から離れる，場合とした．測定は2回行い，上限を60秒としたうちの最大値を解析値とした．

FRは，測定の開始肢位を立位姿勢にて両上肢を肩関節90°屈曲位で保持し，手は軽く握った状態とした．測定手順は，反対側の上肢を下ろした後，足部を動かさずに，もう一方の上肢をできる限り前方へ水平に伸ばすように指示した．その際，第3中手指節関節が開始肢位から水平移動した距離を測定した．測定は2回行い，開始点から最高到達点までの水平距離のうち最高値を解析値とした．

IPSは前後左右4方向へのバランスを反映した指標である[5]．測定機器は，重心動揺計（Gravicorder G-6100, ANIMA）を使用した．測定の開始肢位は足底内側を平行に10 cm離した軽度開脚立位，両上肢下垂位とした．測定の手順は10秒間の静止立位を行い，次に接地面から足底を動かさない状態で身体をできる限り前後左右へ動かし，各位置で10秒間姿勢を保持するように指示した．測定は1回行い，安定域面積と重心動揺面積を用いてIPSを算出した．なお，IPSは log〔(安定域面積＋重心動揺面積)/重心動揺面積〕の式で算出される．安定域面積は前方と後方へ移動した時の各矩形面積の中心を結んだ距離（安定域の前後径）に，右方と左方へ移動した時の各矩形面積の中心を結んだ距離（安定域の左右径）を乗じて求めた．また，重心動揺面積は静止立位時および前後左右移動時の各位置における矩形面積の平均値とした（図1）．

b) 測定時期

臨床的背景因子は入院時，各運動機能は退院時，身体活動は退院後2週間の状態を調査した．

c) 解析および統計学的手法

対象者のうち，65歳未満の患者を壮年群，65歳以上の患者を高齢群とし，臨床的背景因子，各運動機能およ

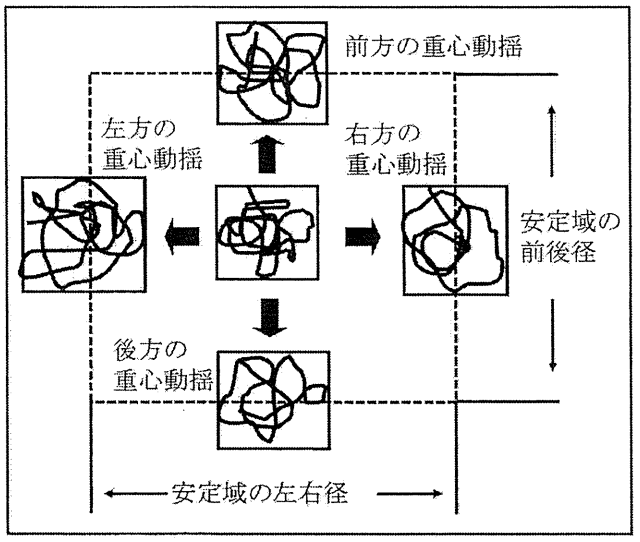

図1　姿勢安定度評価指標（IPS）測定の概念図
重心動揺面積＝実線の5つの矩形面積の平均値，安定域面積＝点線で示す面積，IPS＝log［(安定域面積＋重心動揺面積)/重心動揺面積］

び身体活動を壮年群と高齢群との間で比較した．また，各群において，中強度以上の身体活動時間と臨床的背景因子および各運動機能の関係を検討した．

統計学的手法として，臨床的背景因子，各運動機能および身体活動の2群間の比較にはunpaired-*t*-testを用い，性別および診断名についてはχ^2検定を用いて検討した．また，各群の身体活動と臨床的背景因子および各運動機能の関係はPearsonの積率相関係数を算出した．統計ソフトはSPSS 12.0 J for Windowsを用い，統計学的有意水準は5%未満とした．

4. 結　果

a）臨床的背景因子

臨床的背景因子の結果を壮年群と高齢群に分けて**表1**に示した．年齢およびBNPの項目において高齢群は壮年群と比べて有意に高値を示したが（それぞれ$p < 0.01$），他の項目では両群間に有意な差を認めなかった．

b）身体活動および運動機能

身体活動および運動機能の結果を壮年群と高齢群に分けて**表2**に示した．身体活動についてはすべての項目において両群間に有意な差を認めなかった．一方，運動機能については膝伸展筋力，OLST，FR，およびIPSの項目において，高齢群は壮年群と比べて有意に低値を示した（それぞれ$p < 0.05$, $p < 0.01$, $p < 0.01$, $p < 0.01$）．

表1　IHD患者における壮年群と高齢群の臨床的背景因子

	壮年群 (n＝32)	高齢群 (n＝33)	p値
年　齢（歳）	55.2 ± 8.6	69.5 ± 5.2	＊＊
性　別（男/女）	30/2	29/4	ns
診断名（例）			
急性心筋梗塞	29	30	ns
狭心症	0	1	ns
陳旧性心筋梗塞	3	2	ns
在院日数	24.9 ± 15.1	24.5 ± 11.5	ns
左室駆出率（%）	47.4 ± 10.7	49.1 ± 11.2	ns
BNP（pg/mL）	145.2 ± 251.8	314.5 ± 427.2	＊＊

mean ± SD，BNP：脳性ナトリウム利尿ペプチド
ns：not significant．＊＊：$p < 0.01$

表2　IHD患者における壮年群と高齢群の身体活動および運動機能

	壮年群 (n＝32)	高齢群 (n＝33)	p値
膝伸展筋力（%BW）	50.4 ± 10.5	46.5 ± 12.0	＊
OLST（秒）	57.4 ± 9.6	39.2 ± 23.2	＊＊
FR（cm）	37.2 ± 4.7	33.9 ± 6.3	＊＊
IPS	1.6 ± 0.3	1.4 ± 0.3	＊＊
平均歩数（歩/day）	5847 ± 2977	4823 ± 2823	ns
身体活動時間			
低強度（min）	48.0 ± 18.9	41.0 ± 20.8	ns
中強度以上（min）	15.7 ± 14.7	10.7 ± 11.3	ns

mean ± SD，OLST：片脚立位時間，FR：Functional Reach
IPS：姿勢安定度評価指数，＊：$p < 0.05$，＊＊：$p < 0.01$

c）中強度以上の身体活動時間と臨床的背景因子の関係

両群ともに，中強度以上の身体活動時間と年齢，在院日数，左室駆出率，およびBNPの間に有意な相関は認められなかった．

d）中強度以上の身体活動時間と各運動機能の関係

1）下肢筋力との関係

中強度以上の身体活動時間と下肢筋力の関係を**図2**に示した．壮年群（$r = 0.44$, $p < 0.05$），高齢群（$r = 0.37$, $p < 0.05$）ともに，中強度以上の身体活動時間と下肢筋力の間に有意な正の相関が認められた．

2）静的バランスとの関係

中強度以上の身体活動時間と静的バランスの関係を**図3**に示した．壮年群では中強度以上の身体活動時間とOLSTの間に有意な相関は認められなかったのに対し，高齢群では有意な正の相関が認められた（$r = 0.47$, $p < 0.05$）．

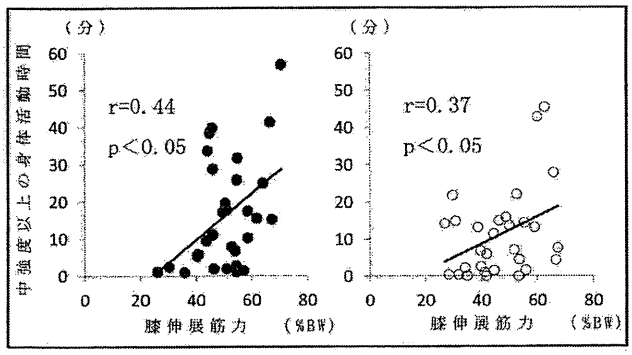

図2　中強度以上の身体活動時間と下肢筋力の関係
●：壮年群　○：高齢群
両群ともに，中強度以上の身体活動時間と下肢筋力の間に有意な正の相関が認められた．

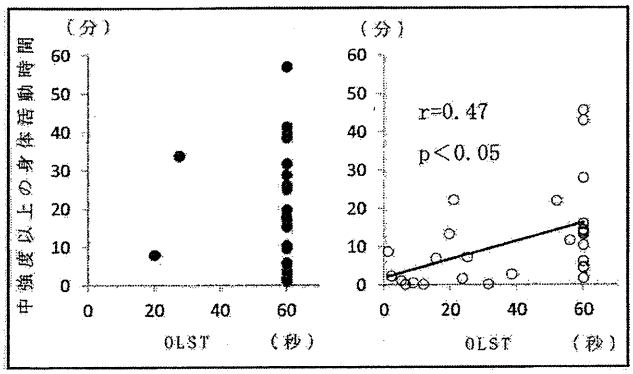

図3　中強度以上の身体活動時間と静的バランスの関係
●：壮年群　○：高齢群　OLST：片脚立位時間
壮年群では中強度以上の身体活動時間とOLSTの間に有意な相関は認められなかったのに対し，高齢群では有意な正の相関が認められた．

3）動的バランスとの関係

中強度以上の身体活動時間と動的バランスの関係を図4に示した．壮年群では中強度以上の身体活動時間とFRおよびIPSの間に有意な相関は認められなかったのに対し，高齢群では，中強度以上の身体活動時間とFR（$r = 0.43$，$p < 0.05$）およびIPS（$r = 0.41$，$p < 0.05$）の間に有意な正の相関が認められた．

5. 考察

IHD患者に対する運動療法は急性期治療後の日常生活活動の再獲得や運動耐容能の改善だけでなく，回復期以降の再発予防においても重要な役割を果たしている[1]．一方，回復期以降の身体活動量を高く維持することは，心血管イベントの再発予防に寄与することが知られている．また，身体活動の強度を中強度以上に保つことで，再発率はさらに減少すると報告されている[2]．このため，退院時ならびに退院後の運動指導をより具体的

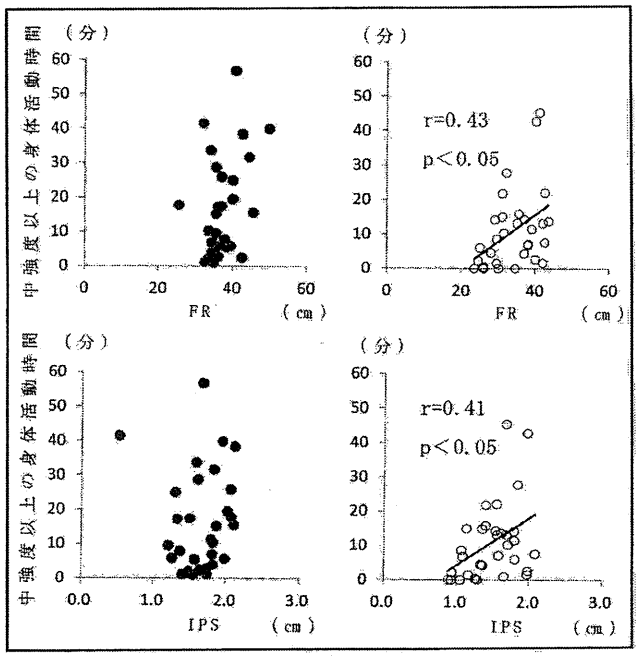

図4　中強度以上の身体活動時間と動的バランスの関係
●：壮年群　○：高齢群　FR：Functional Reach
IPS：姿勢安定度評価指標
壮年群では中強度以上の身体活動時間とFRおよびIPSの間に有意な相関は認められなかったのに対し，高齢群では有意な正の相関が認められた．

に進めていく必要がある．そこで本研究は，IHD患者の退院後の身体活動のなかでも中強度以上の身体活動時間に注目し，退院時の運動機能との関連を検討した．さらに高齢心疾患患者の運動機能は壮年者と比べて低下している[3]ことから，本研究では中強度以上の身体活動時間と運動機能との関係を壮年者と高齢者に分けて検討することにした．すると，壮年群では中強度以上の身体活動時間には下肢筋力のみが関与していたのに対して，高齢群では下肢筋力に加えて静的と動的バランス機能の両方が関与していることが認められた．山本ら[6]の報告によると，入院期の高齢心疾患患者のバランス機能は壮年者に比べて低下しており，そのバランス機能の低下は心疾患の既往のない地域在住高齢者を対象とした先行研究と同様に，歩行速度と密接に関連していることが認められている．本研究の対象者が心疾患であり，退院後の身体活動として走行や激しいスポーツは制限されていたことを考えると，身体活動量の測定で用いたライフコーダに内蔵されている加速度センサーが感知した強度は主に歩行速度を反映していたと思われる．このため，退院時の高齢IHD患者の中強度以上の身体活動時間には下肢筋力だけでなく，静的ならびに動的バランス機能が関連したと考えられた．なお，両群ともに中強度以上の身体

活動時間と左室駆出率およびBNPの間に関連が認められなかったこと，また高齢者のBNPが壮年者に比べて高値を示したにもかかわらず，中強度以上の身体活動時間自体に両群間に差異が認められなかったことを考え合わせると，今回の対象者においては，退院後の身体活動の制限に心機能の重症度が関係している可能性は低いと考えられた．

以上のことから，高齢IHD患者の退院後の身体活動の運動強度を中強度以上に保つためには，下肢筋力だけでなくバランス機能を改善させる必要があると考えられる．とくに，通常の有酸素運動や筋力トレーニングを主体とした運動療法では，高齢IHD患者のバランス機能の回復は遅延する[7]ことから，高齢IHD患者に対しては，バランス機能を改善するための特定な運動療法ならびに運動指導を優先的に実施する必要があると思われた．

なお，本研究の限界として，身体活動量の測定時期が退院後2週間以内であったため，医療者側からの治療指針の一環として，スポーツなどの運動強度の高い活動は一律に制限されていた点が挙げられる．壮年者と高齢者の間に中強度以上の身体活動時間の違いがみられなかった理由も，この測定時期が影響している可能性がある．このため，今後は退院後3ヵ月あるいは6ヵ月以降において同調査を行い，身体活動と運動機能との関連を検討する必要があると思われた．また，本研究は観察研究であるため，高齢IHD患者のバランス機能を改善することが，中強度の身体活動時間の増大につながるか否かは明らかでない．このため，高齢IHD患者に対して，バランス機能に対する特定な運動指導を実施する介入研究を行い，中強度の身体活動時間に与える影響を検討する必要があると思われた．

6. 結　論

高齢IHD患者の退院後の中強度以上の身体活動時間は壮年心疾患患者とは異なり，下肢筋力に加えてバランス機能が影響していた．このため，高齢IHD患者の運動指導の際には，下肢筋力に加えてバランス機能を評価し，バランス機能に対する特定な運動指導を実施する必要性が示唆された．

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Usefulness of Pet Ownership as a Modulator of Cardiac Autonomic Imbalance in Patients With Diabetes Mellitus, Hypertension, and/or Hyperlipidemia

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Among patients with coronary artery disease, pet owners exhibit a greater 1-year survival rate than nonowners. Lifestyle-related diseases are well-known risk factors for coronary artery disease and induce imbalances in autonomic nervous activity. The purpose of the present study was to determine whether pet ownership modulates cardiac autonomic nervous activity imbalance in patients with lifestyle-related diseases such as diabetes mellitus, hypertension, and hyperlipidemia. A total of 191 patients (mean age 69 ± 8 years) were interviewed about their pet ownership status and were classified into pet owner and nonowner groups. After recording a 24-hour Holter electrocardiogram for heart rate variability analysis, frequency-domain and nonlinear-domain analyses were performed to determine the high-frequency (HF) and low-frequency (LF) components, LF/HF ratio, and entropy. The heart rate variability parameters were assessed for 24 hours, during the day (8.00 A.M. to 5.00 P.M.), and during the night (0:00 A.M. to 6.00 A.M.), and compared between the 2 groups. To evaluate the potential predictive factors for cardiac autonomic imbalance, univariate and multivariate analyses of HF and LF/HF were conducted for potential confounding variables. The pet owner group exhibited significantly greater HF_{24h}, HF_{day}, HF_{night}, $entropy_{24h}$, $entropy_{day}$, and $entropy_{night}$ and significantly lower LF/HF_{24h} and LF/HF_{night} compared to the nonowner group. On multivariate analysis, pet ownership was independently and positively associated with HF_{24h}, HF_{day}, and HF_{night} and inversely associated with LF/HF_{24h} and LF/HF_{night}. In conclusion, these results suggest that pet ownership is an independent modulator of cardiac autonomic imbalance in patients with lifestyle-related diseases. **(Am J Cardiol 2012;109:1164–1170)**

Lifestyle-related diseases, such as diabetes mellitus, hypertension, and hyperlipidemia, are well-known risk factors for coronary artery diseases.[1,2] Moreover, patients with lifestyle-related diseases exhibit autonomic nervous activity imbalance.[2–5] The effect of pet ownership on cardiac autonomic nervous activity has not been evaluated in patients with lifestyle-related diseases. Accordingly, the aim of the present study was to determine whether pet ownership modulates cardiac autonomic nervous activity imbalances in patients with lifestyle-related diseases.

Methods

The Ethics Committee on Human Research of Kitasato University approved the present study. Outpatients who regularly visited the cardiovascular center of Kitasato University Hospital from January 2009 to December 2010 because of diabetes mellitus, hypertension, and hyperlipidemia were eligible to participate. Patients with other major illnesses, such as old myocardial infarction, angina pectoris, chronic renal failure, and frequent arrhythmias, were excluded. A total of 244 patients who satisfied the initial criteria were informed about the study, and all patients provided written informed consent.

The patients were classified into 2 groups according to pet ownership: pet owner and nonowner groups. A pet owner was defined as a patient who currently had a pet and had had the pet for >6 months at study enrollment. Those patients who had had pets in the past, but not currently, were excluded from the present study. In the pet owner group, the patients were interviewed about their pet ownership status, including the number and species of pets, pet-keeping duration, where the pet is kept (inside or outside the house), the amount of time spent a day by the owners walking their dogs, and whether the patient was the main caretaker of the pet. Pet ownership status is listed in Table 1.

We assessed the disease-specific cardiovascular functional status using the specific activity scale. The specific activity scale included 21 items of self-reported data on the performance of well-defined daily activities (e.g., walking, climbing stairs, showering, dressing), which was developed

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Table 1
Pet ownership status

Variable	Dog Owners (n = 46)	Cat Owners (n = 27)	Other Owners* (n = 9)
Pet-keeping duration (years)	15 ± 11	16 ± 12	15 ± 8
Keeping area			
Inside	35	25	6
Outside	11	2	3
Main caretaker			
Yes	17	17	4
No	23	3	2
Do not know	6	7	3
Walking with dog			
Yes	29	—	—
No	17		
Amount of time spent with dog walking per day			
<30 minutes	6	—	—
≥30 minutes	12	—	—
≥60 minutes	8	—	—
≥120 minutes	3	—	—

Data are presented as mean ± SD.
* Fish, bird, and turtle.

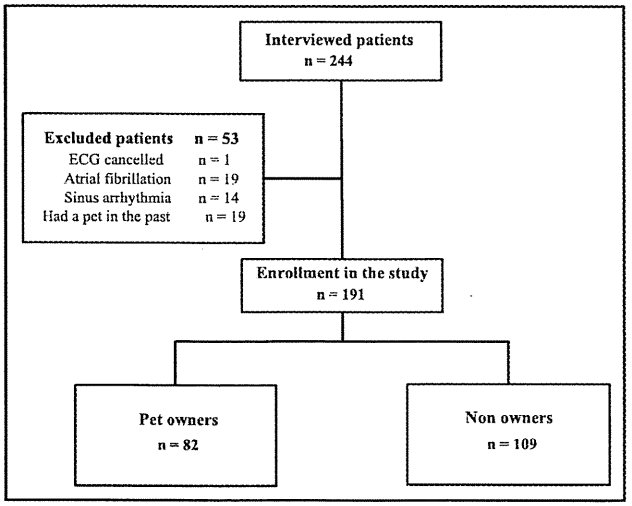

Figure 1. Patient flow charts.

as an alternative to the New York Heart Association functional classification.[6]

After the interview, the patients underwent physical and blood examinations, 24-hour Holter electrocardiogram, echocardiogram, and a measurement of brachial-ankle pulsewave velocity.[7] After the 24-hour Holter electrocardiogram, the patients who exhibited atrial fibrillation, marked sinus arrhythmia, or frequent atrial or ventricular premature beats were excluded. Thus, 191 patients participated in the present study (Figure 1).

The clinical and sociodemographic variables, including gender, age, systolic blood pressure, diastolic blood pressure, heart rate, diagnosis, current smoking status, and medications, were obtained from the medical records at the beginning of the study. The routine biochemical parameters, such as the serum concentrations of low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, and hemoglobin A1c, were evaluated. The body mass index was calculated as the body weight in kilograms divided by height in square meters. Echocardiographic analysis was performed by an investigator who was unaware of the clinical and treatment status of patients. Left atrial diameter, left ventricular end-diastolic diameter, left ventricular end-systolic diameter, left ventricular posterior wall thickness, and interventricular septal thickness were measured to calculate the left ventricular ejection fraction and left ventricular muscle mass.[8] The ventricular muscle mass was corrected by body surface area and expressed as the ventricular muscle mass index.[8]

Autonomic nervous activity was evaluated by heart rate variability (HRV) analysis using a 24-hour Holter electrocardiogram (Aria, Del Mar Reynolds Medical, Irvine, California). The HRV based on the beat-to-beat RR intervals of normal sinus beats was assessed for 24 hours, during the day (8.00 A.M. to 5.00 P.M.) and during the night (0:00 A.M. to 6.00 A.M.). A Holter recording was considered suitable for time-domain, frequency-domain, or nonlinear-domain HRV analysis if it contained ≥95% analyzable data for each period.

The time-domain indexes included the standard deviation of a series of all-normal RR intervals (SDNN), standard deviation of mean RR intervals of a 5-minute electrocardiogram (SDANN), the square root of the average of squares of differences between consecutive RR intervals (RMSSD), and the percentage of RR intervals that differed from each other by >50 ms. SDNN and SDANN reflect overall HRV.[9,10] RMSSD and percentage of RR intervals that differed from each other by >50 ms reflect the cardiac parasympathetic nervous activity.[9,10] The time-domain parameter indexes for the 24-hour, daytime, and nighttime periods are presented as the $SDNN_{24h}$, $SDNN_{day}$, $SDNN_{night}$, $RMSSD_{24h}$, $RMSSD_{day}$, $RMSSD_{night}$, $SDANN_{24h}$, $SDANN_{day}$, and $SDANN_{night}$, respectively.

Using RR intervals obtained from a 24-hour Holter electrocardiogram, a beat-to-beat spectral analysis was performed with a combination of the maximum entropy method for spectral analysis and the nonlinear least squares method for fitting analysis[11,12] (MemCalc, GMS, Tokyo, Japan) to obtain the HR and 2 frequency bands: a low frequency (LF) component of 0.04 to 0.15 Hz and a high frequency (HF) component of 0.15 to 0.4 Hz. The HF and LF/HF ratio indicate parasympathetic nervous activity and the dominance of sympathetic nervous activity over parasympathetic activity, respectively.[13] The HR and frequency-domain parameters for the different periods are presented as HR_{24h}, HR_{day}, HR_{night}, HF_{24h}, HF_{day}, HF_{night}, LF/HF_{24h}, LF/HF_{day}, and LF/HF_{night}, respectively.

A nonlinear-domain analysis of HRV was also performed with MemCalc to assess entropy as a complexity of the cardiovascular system. Entropy was calculated from a pulse time series of 4 RR intervals and expressed as a scale from 0% (indicating no HRV randomness) to 100% (indicating complete HRV randomness).[11,14] Entropy for the different periods is presented as $entropy_{24h}$, $entropy_{day}$, and $entropy_{night}$, respectively.

The Mann-Whitney *U* test, Student's *t* test, and chi-square test were used to examine the differences in the

Table 2
Patient characteristics

Characteristic	Total (n = 191)	Pet Owner		P Value
		Yes (n = 82)	No (n = 109)	
Men	143 (75%)	60 (73%)	83 (76%)	.38
Age (years)	69 ± 8	68 ± 10	70 ± 7	.51
Systolic blood pressure (mm Hg)	126 ± 15	125 ± 15	126 ± 16	.73
Diastolic blood pressure (mm Hg)	70 ± 11	70 ± 9.7	71 ± 11	.23
Heart rate (beats/min)	68 ± 8	67 ± 8	69 ± 9	.10
Body mass index (kg/m^2)	23.8 ± 3	23.9 ± 3	23.8 ± 3	.79
Low-density lipoprotein cholesterol (mg/dL)	108 ± 26	110 ± 25	107 ± 27	.11
High-density lipoprotein cholesterol (mg/dL)	57 ± 19	57 ± 16	57 ± 21	.77
Triglycerides (mg/dL)				.89
Median	112	109	113	
Interquartile range	74–162	71–163	76–161	
Hemoglobin A1c (%)	5.9 ± 0.9	5.9 ± 0.8	6.0 ± 1	.68
Left ventricular ejection fraction (%)	62 ± 7.7	61 ± 8	62 ± 8	.60
Left ventricular muscle mass index	126 ± 29	127 ± 27	125 ± 31	.40
Brachial ankle pulse wave velosity (cm/s)				.49
Median	1,620	1,619	1,614	
Interquartile range	1,421–1,763	1,393–1,759	1,447–1,826	
Diagnosis				
Diabetes mellitus	87 (46%)	38 (46%)	49 (45%)	.48
Hypertension	90 (47%)	41 (50%)	49 (45%)	.29
Hyperlipidemia	117 (61%)	47 (57%)	70 (64%)	.21
Current smoker	21 (11%)	11 (13%)	10 (9%)	.24
Antidiabetic agents				
Insulin	7 (8%)	2 (5%)	5 (10%)	.57
Hypoglycemic agents	29 (33%)	12 (32%)	17 (35%)	.43
Diet therapy	51 (59%)	24 (63%)	27 (55%)	.51
Medication use				
β Blockers	88 (46%)	39 (48%)	49 (45%)	.42
Statins	105 (55%)	45 (55%)	60 (55%)	.55
Specific activity scale (METs)	6.7 ± 0.9	6.6 ± 0.86	6.7 ± 1	.86

Data are presented as mean ± SD, n (%), or median and interquartile range (25–75%).
METs = metabolic equivalent (1 MET = 3.5 mL/kg/min).

clinical characteristics and autonomic nervous activity between pet owner and nonowner groups. The comparisons of the mean values for normally distributed continuous variables were performed using Student's *t* test. For continuous variables with non-normal distributions, the Mann-Whitney *U* test was used. Univariate and multivariate linear regression analyses of the HF and LF/HF measurements were conducted for potential confounding variables: gender, age, HR, body mass index, left ventricular ejection fraction, brachial-ankle pulsewave velocity, diabetes mellitus, hypertension, hyperlipidemia, smoking status, and pet ownership, to evaluate potential predictive factors for autonomic nervous activity in patients with lifestyle-related diseases. $p < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS, versions 16.0J for Windows (SPSS Japan, Tokyo, Japan).

Results

The patient characteristics are presented in Table 2. No significant differences were found in the demographic and physiologic characteristics between the pet owner and nonowner groups.

The HR and time-domain analysis of HRV parameters are presented in Table 3. HR_{night} was significantly lower in the pet owner group than in the nonowner group ($p < 0.05$). $RMSSD_{24h}$, $RMSSD_{day}$, $RMSSD_{night}$, and percentage of RR intervals that differed from each other by >50 ms were significantly greater in the pet owner group than in the nonowner group ($p < 0.01$ for each). HF_{24h}, HF_{day}, and HF_{night} were significantly greater in the pet owner group than in the nonowner group ($p < 0.01$ for each; Figure 2). The LF/HF_{24h} and LF/HF_{night} were significantly lower in the pet owner group than in the nonowner group ($p < 0.05$ for each; Figure 3); and $entropy_{24h}$, $entropy_{day}$, and $entropy_{night}$ were significantly greater in the pet owner group than in the nonowner group ($p < 0.01$ for each; Figure 4).

The univariate and multivariate regression analysis results of HF are presented in Table 4. On multivariate analysis, pet ownership was positively associated with HF_{24h}, HF_{day}, and HF_{night} ($p < 0.01$ for each), and HR was inversely associated with HF_{24h}, HF_{day}, and HF_{night} ($p < 0.01$, $p < 0.05$, and $p < 0.01$, respectively). Diabetes mellitus was inversely associated with HF_{24h} and HF_{day} ($p < 0.01$ for each), and hyperlipidemia was inversely associated with HF_{24h}, HF_{day}, and HF_{night} ($p < 0.01$ for each).

The univariate and multivariate regression analysis results of LF/HF are presented in Table 5. On multivariate analysis, pet ownership was inversely associated with LF/

Table 3
Heart rate and heart rate variability

Variable	Pet Owners	Nonowners	p Value
Heart rate			
24 hour	67 ± 8	69 ± 9	0.10
Day	70 ± 10	73 ± 10	0.14
Night	59 ± 8	62 ± 9	0.05*
Mean NN (ms)	874 (772–957)	837 (769–937)	0.14
pNN50 (%)	5.2 (1.4–9.9)	2.5 (0.9–5.8)	0.001†
$SDNN_{24h}$ (ms)	131 (109–153)	119 (102–141)	0.07
$SDNN_{day}$ (ms)	106 (84–127)	102 (84–118)	0.19
$SDNN_{night}$ (ms)	84 (71–111)	82 (69–98)	0.11
$RMSSD_{24h}$ (ms)	28 (21–37)	24 (18–29)	0.001†
$RMSSD_{day}$ (ms)	25 (20–34)	22 (17–28)	0.002†
$RMSSD_{night}$ (ms)	31 (22–38)	24 (19–31)	0.003†
$SDANN_{24h}$ (ms)	122 (102–142)	111 (94–133)	0.06
$SDANN_{day}$ (ms)	97 (78–118)	95 (77–110)	0.18
$SDANN_{night}$ (ms)	69 (57–91)	64 (53–81)	0.11

Data are presented as mean ± SD or median (interquartile range, 25–75%).

* $p < 0.05$; † $p < 0.01$, pet owner group vs nonowner group.

Mean NN = mean of RR intervals; pNN50 = percentage of RR intervals that differ each other >50 ms; RMSSD = square root of mean of sum of squares of differences between consecutive RR intervals; SDANN = standard deviation of mean RR intervals in all 5-minute segments of entire recording; SDNN = standard deviation of all RR intervals.

HF_{24h} and LF/HF_{night} ($p < 0.05$ for each). Additionally, gender was inversely associated with LF/HF_{night} ($p < 0.01$); age was inversely associated with LF/HF_{24h}, LF/HF_{day}, and LF/HF_{night} ($p < 0.01$ for each); HR was positively associated with LF/HF_{night} ($p < 0.05$); left ventricular ejection fraction was positively associated with LF/HF_{24h}, LF/HF_{day}, and LF/HF_{night} ($p < 0.01$ for each); hyperlipidemia was positively associated with LF/HF_{day} ($p < 0.05$); and smoking status was inversely associated with LF/HF_{night} ($p < 0.01$).

Discussion

In the present study, it was determined that the comparison of autonomic nervous activity between pet owner and nonowner groups was statistically appropriate, given the lack of a significant difference in the baseline characteristics (e.g., clinical, sociodemographic, and cardiac function) among the 2 groups.

Consistent with our hypothesis, pet ownership was closely associated with the modulation of cardiac autonomic nervous activity in patients with lifestyle-related diseases.

Friedmann et al[15,16] reported that pet ownership is associated with a greater 1-year survival rate after hospital discharge in patients with heart failure. In a study of subset data from their study, RMSSD in HRV was significantly greater in pet owners than in nonowners among patients with old myocardial infarction.[17] In the present study, the pet owners had significantly greater percentage of RR intervals that differed from each other by >50 ms and RMSSD than nonowners, consistent with the results reported by Friedmann et al.[17] We also showed significantly greater HF and significantly lower LF/HF and HR_{night} in pet owners than in nonowners among patients with lifestyle-related diseases. These findings suggest that pet owners had greater elevated parasympathetic and diminished sympathetic nervous activities than nonowners, indicating that pet ownership attenuated the imbalance in autonomic nervous activity among patients with lifestyle-related diseases, resulting in a decreased nighttime HR among pet owners. In a nonlinear analysis, several reports have shown that entropy reflects the total fluctuation in HR, thereby indicating the complexity of HRV, and that greater entropy indicates a superior capability to modulate perturbations in the cardiovascular system.[14] Furthermore, a previous study reported that among patients who underwent transurethral surgery, those with greater entropy exhibited a smaller decrease in systolic blood pressure and lower incidence of hypotension during spinal anesthesia than those with lower entropy.[18] In the present study, pet owners had significantly greater entropy than nonowners. Thus, pet owners exhibited greater HRV complexity, indicating a greater adaptability to perturbations in the cardiovascular system.

Lifestyle-related diseases, which are risk factors for coronary artery disease, have been reported to diminish HRV and induce autonomic nervous activity imbalance.[2–5] It is also well known that patients with coronary artery disease have lower HRV, indicating greater mortality or a poor prognosis.[13,19–21] Multivariate analysis in the present study revealed that pet ownership was independently and positively associated with parasympathetic nervous activity and inversely with sympathetic nervous activity. These findings confirmed pet ownership as an independent factor for modulating autonomic nervous activity in patients with lifestyle-related diseases.

A few limitations of the present study are worth noting. Because the present study was a cross-sectional study, additional longitudinal research is required to clarify the effects of pet ownership on preventing coronary artery disease. In analyzing HRV results, factors such as daily walking should be considered in future studies, given reports that the mere routine of daily walking ameliorates autonomic nervous activity imbalance.[22] Furthermore, the differences in pet ownership status could affect autonomic nervous activity. Motooka et al[23] reported increased parasympathetic nervous activity in healthy seniors while walking with a dog. Accordingly, the HRV results should be compared between pet owners, particularly dog owners, with and without daily walking. However, when pet owners were classified into subgroups according to pet ownership status, the sample size in each subgroup was too small to compare the autonomic nervous activity between the subgroups. Pet ownership status, including the number and species of pets, duration of pet keeping, where the pet is kept (inside or outside the house), amount of time spent a day by owners walking their dogs, and whether the subject was the main caretaker of the pet, should be considered in future analyses to fully elucidate the interaction between pet owners and pets.

Acknowledgment: We thank Keiko Miyashita, CT and Tomomi Sakamoto, CT for the electrocardiographic data analysis.

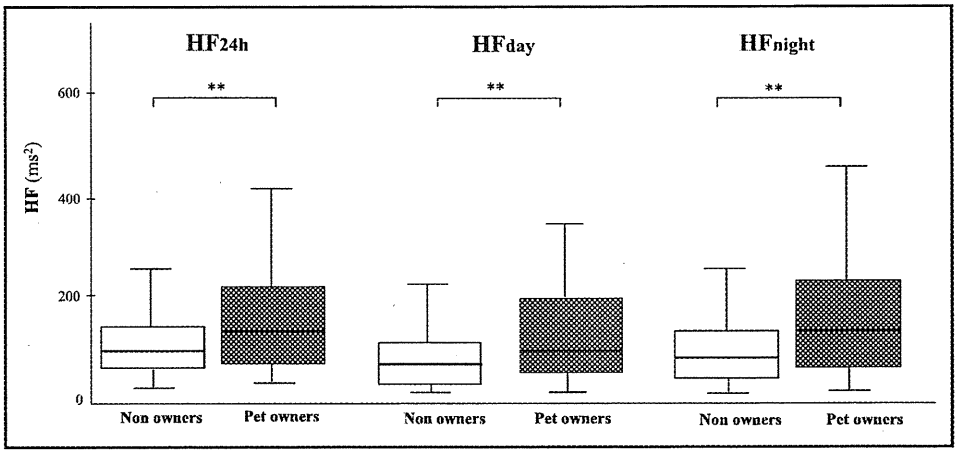

Figure 2. HF component in pet owner and nonowner groups. Data are presented as box plots displaying median, 25th and 75th percentiles (boxes), and 10th and 90th percentiles (whiskers). *p <0.05 and **p <0.01 (pet owner vs nonowner groups).

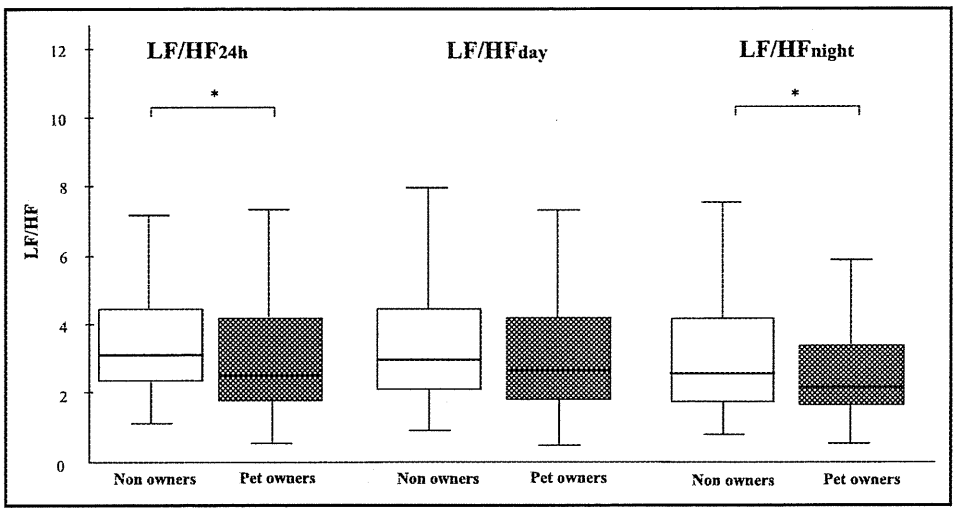

Figure 3. LF/HF in pet owner and nonowner groups. Data are presented as box plots displaying median, 25th and 75th percentiles (boxes), and 10th and 90th percentiles (whiskers). *p <0.05 (pet owner vs nonowner groups).

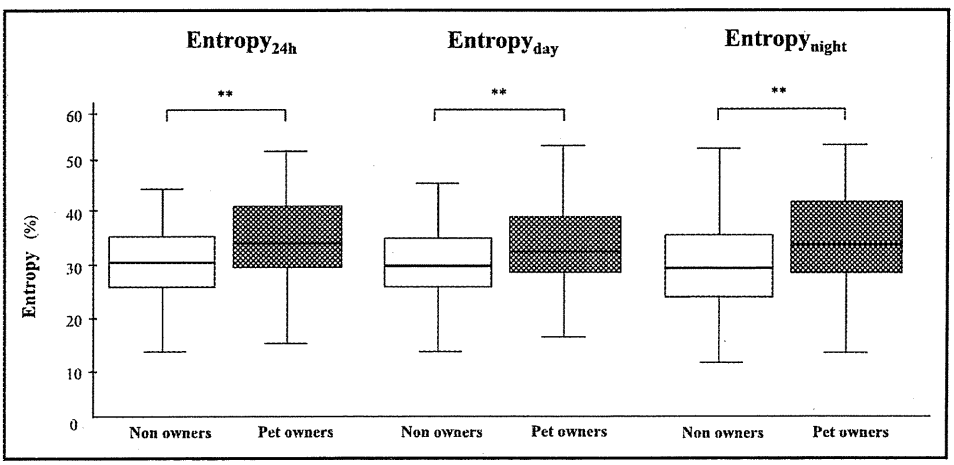

Figure 4. Entropy in pet owner and nonowner groups. Data are presented as box plots displaying median, 25th and 75th percentiles (boxes), and 10th and 90th percentiles (whiskers). *p <0.05 and **p <0.01 (pet owner vs nonowner groups).

Table 4
Clinical predictors for high frequency (HF) in heart rate variability (HRV) using uni- and multivariate linear regression analyses

High Frequency	Univariate			Multivariate		
	B	95% CI	p Value	B	95% CI	p Value
24 hours						
Gender	7.4	−27.1, 41.9	0.67	8.2	−24.9, 41.3	0.63
Age	−0.79	−2.6, 1.0	0.38	−0.56	−2.4, 1.3	0.55
Heart rate	−2.9	−4.6, −1.2	0.001*	−2.55	−4.2, −0.9	0.003*
Body mass index	1.3	−3.6, 6.3	0.60	2.2	−2.5, 6.9	0.35
Left ventricular ejection fraction	−0.04	−2.0, 1.9	0.97	−0.4	−2.24, 1.5	0.70
Brachial ankle pulse wave velosity	−0.04	−0.09, 0.006	0.09	−0.03	−0.08, 0.02	0.27
Diabetes mellitus	−30.53	−60.3, −0.8	0.044†	−38.3	−67, −9.8	0.009*
Hypertension	12.5	−17.4, 42.4	0.41	18.6	−11.6, 48.8	0.24
Hyperlipidemia	−44.2	−74.3, −14.1	0.004*	−40.5	−69.2, −11.9	0.006*
Smoking	5.2	−42.6, 53.0	0.83	7.9	−37.9, 53.6	0.74
Pet ownership	66	37.3, 94.8	0.00*	54.0	25.8, 82.1	0.000*
Day						
Gender	4.0	−31.0, 39.1	0.82	0.9	−34.0, 35.8	0.96
Age	−0.3	−2.1, 1.5	0.73	−0.24	−2.2, 1.7	0.81
Heart rate	−2.5	−4.3, −0.8	0.005*	−1.9	−3.7, 0.09	0.04†
Body mass index	1.1	−4.0, 6.2	0.66	2.2	−2.8, 7.1	0.39
Left ventricular ejection fraction	0.52	−1.5, 2.5	0.61	0.28	−1.7, 2.2	0.78
Brachial ankle pulse wave velosity	−0.03	−0.08, 0.02	0.26	−0.02	−0.08, 0.03	0.38
Diabetes mellitus	−29	−59.3, 1.2	0.06	−38.1	−68, −8.1	0.01*
Hypertension	17.5	−12.9, 47.8	0.26	22.5	−9.3, 54.3	0.16
Hyperlipidemia	−46.2	−76.6, −15.7	0.003*	−42.7	−72.9, −12.6	0.006*
Smoking	1.2	−47.4, 49.7	1.0	3.3	−44.9, 51.5	0.89
Pet ownership	52.1	22.3, 81.9	0.001*	42.56	12.93, 72.2	0.005*
Night						
Gender	32.1	−14.5, 78.6	0.18	35.08	−10.2, 80.4	0.13
Age	−2.1	−4.5, 0.31	0.09	−1.74	−4.3, 0.81	0.18
Heart rate	−4.1	−6.4, −1.7	0.001*	−3.55	−5.9, −1.2	0.003*
Body mass index	2.6	−4.1, 9.4	0.45	3.05	−3.3, 9.4	0.35
Left ventricular ejection fraction	0.1	−2.5, 2.8	0.91	−0.72	−3.3, 1.8	0.58
Brachial ankle pulse wave velosity	−0.06	−0.13, 0.003	0.06	−0.04	−0.1, 0.03	0.30
Diabetes mellitus	−19.1	−59.7, 21.6	0.36	−28.5	−67.5, 10.5	0.15
Hypertension	18.0	−22.6, 58.5	0.38	25.64	−15.7, 67.0	0.22
Hyperlipidemia	−57.7	−98.6, −16.9	0.006*	−51.57	−90.8, −12.4	0.01*
Smoking	18.5	−46.4, 83.3	0.58	20.17	−42.5, 82.8	0.53
Pet ownership	84.6	45.5, 123.8	0.00*	65.3	26.8, 103.8	0.001*

Multivariate linear regression included all univariable predictors.
* $p < 0.01$; † $p < 0.05$.
B = regression coefficient; CI = confidence interval.

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Table 5
Clinical predictors for low frequency (LF)/high frequency (HF) ratio in heart rate variability (HRV) using uni- and mutivariate linear regression analyses

Low/High Frequency Ratio	Univariate			Multivariate		
	B	95% CI	p Value	B	95% CI	p Value
24 hours						
Gender	−0.23	−0.9, 0.4	0.50	−0.61	−1.2, 0.02	0.11
Age	−0.09	−0.12, −0.05	0.00*	−0.08	−0.12, −0.04	0.00*
Heart rate	0.04	0.00, 0.07	0.047†	0.02	−0.01, 0.06	0.13
Body mass index	0.04	−0.05, 0.14	0.37	0.01	−0.08, 0.1	0.79
Left ventricular ejection fraction	0.06	0.02, 0.1	0.003*	0.06	0.03, 0.1	0.001*
Brachial ankle pulse wave velosity	−0.001	−0.002, −0.00	0.001*	0	−0.002, 0.00	0.27
Diabetes mellitus	−0.04	−0.6, 0.5	0.90	0.06	−0.5, 0.6	0.82
Hypertension	−0.8	−1.4, −0.2	0.006*	−0.29	−0.9, 0.29	0.33
Hyperlipidemia	0.53	−0.06, 1.1	0.08	0.45	−0.1, 1.0	0.11
Smoking	0.23	−0.7, 1.2	0.63	−0.57	−1.4, 0.3	0.20
Pet ownership	−0.6	−1.2, −0.01	0.045†	−0.60	−1.13, −0.07	0.028†
Day						
Gender	0.11	−0.56, 0.77	0.75	−0.21	−0.8, 0.42	0.52
Age	−0.1	−0.13, −0.07	0.00*	−0.08	−0.12, −0.05	0.00*
Heart rate	0.03	−0.002, 0.07	0.07	0.02	−0.02, 0.05	0.31
Body mass index	0.05	−0.05, 0.14	0.36	0.01	−0.08, 0.1	0.76
Left ventricular ejection fraction	0.06	0.02, 0.1	0.003*	0.06	0.02, 0.09	0.002*
Brachial ankle pulse wave velosity	−0.002	−0.003, −0.00	0.000*	0	−0.001, 0	0.26
Diabetes mellitus	−0.08	−0.66, 0.5	0.78	0.01	−0.52, 0.55	0.96
Hypertension	−0.82	−1.4, −0.25	0.005*	−0.26	−0.83, 0.31	0.37
Hyperlipidemia	0.60	0.02, 1.2	0.044†	0.54	0.002, 1.1	0.049†
Smoking	0.56	−0.36, 1.48	0.23	−0.18	−1.04, 0.69	0.69
Pet ownership	−0.39	−1.0, 0.2	0.19	−0.44	−0.98, 0.09	0.10
Night						
Gender	−0.78	−1.5, 0.05	0.036†	−1.3	−1.98, −0.57	0.00*
Age	−0.07	−0.1, −0.03	0.001*	−0.07	−0.11, −0.03	0.001*
Heart rate	0.04	0.003, 0.08	0.034†	0.04	0.00, 0.07	0.05†
Body mass index	0.03	−0.07, 0.14	0.55	0.00	−0.1, 0.1	0.94
Left ventricular ejection fraction	0.05	0.01, 0.1	0.013†	0.07	0.03, 0.11	0.001*
Brachial ankle pulse wave velosity	−0.001	−0.002, 0.00	0.05	0	−0.001, 0.001	0.52
Diabetes mellitus	0.1	−0.55, 0.74	0.77	0.20	−0.41, 0.81	0.51
Hypertension	−0.8	−1.4, −0.12	0.02†	−0.35	−1.0, 0.3	0.28
Hyperlipidemia	0.19	−0.46, 0.85	0.56	0.08	−0.53, 0.69	0.80
Smoking	−0.5	−1.5, 0.57	0.39	−1.34	−2.3, −0.36	0.008*
Pet ownership	−0.83	−1.46, −0.19	0.011†	−0.8	−1.3, −0.15	0.015†

Multivariate linear regression included all univariable predictors.
* $p < 0.01$; † $p < 0.05$.
B = regression coefficient; CI = confidence interval.

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Ezetimibe and Reactive Oxygen Species

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Abstract: Ezetimibe is a potent inhibitor of cholesterol absorption that has been approved for the treatment of hypercholesterolemia. Statin, 3-hydroxy-3 methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, is an inhibitor of cholesterol synthesis. Statin is the first-choice drug to reduce low-density lipoprotein (LDL)-cholesterol for patients with hypercholesterolemia, due to its strong effect to lower the circulating LDL-cholesterol levels. Because a high dose of statins causes concern about rhabdomyolysis, it is sometimes difficult to achieve the guideline-recommended levels of LDL-cholesterol in patients with high LDL-cholesterol treated with statin monotherapy. Ezetimibe has been reported to reduce LDL-cholesterol safely with both monotherapy and combination therapy with statins. Ezetimibe is especially expected to be the best pharmacological option for the treatment of patients unable to achieve LDL-cholesterol goals with statins. Reactive oxygen species (ROS) are produced at low levels to maintain physiological redox balance. Oxidative stress results when ROS production exceeds the ability of cells to detoxify ROS. Overproduction of ROS damages cellular components, including lipids, leading to decline in physiological function and cell death. Oxidative stress exacerbates atherosclerosis, the major risk factor for coronary artery disease and ischemic stroke, at every step involves the accumulation of oxidized LDL in the arteries, leading to foam cell formation, plaque development, and plaque rupture. This review focuses on the recent findings of ezetimibe-related atheroprotective effects in vasculature. Moreover, known and proposed mechanisms of how ezetimibe could improve ROS-induced pro-atherosclerotic conditions in vasculature are discussed; these effects may help to explain the mechanisms by which ezetimibe may protect vascular from atherosclerosis.

Keywords: Reactive oxygen species, atherosclerosis, dyslipidemia, inflammation, cholesterol.

1. INTRODUCTION

Atherosclerosis is the most common pathological process that leads to cardiovascular diseases, a disease of large- and medium-sized arteries that is characterized by formation of atherosclerotic plaques consisting of necrotic cores, calcified regions, accumulated modified lipids, inflamed endothelial cells (ECs), smooth muscle cells, leukocytes, and foam cells [1]. Although low-density lipoproteins (LDL) remain the most important and powerful risk factor for atherosclerosis, vascular inflammation- and oxidative stress-induced mechanisms of atherosclerosis have gained tremendous interest in the last two decades [1-4]. LDL are susceptible to structural modifications by oxidation, particular, particularly the small dense LDL particles [5]. Under proatherogenic conditions, nitric oxide production from ECs is reduced and the burden of reactive oxygen species (ROS) is increased [6, 7].

Ezetimibe is an epoch-making cholesterol transporter inhibitor in the small intestine to treat dyslipidemia patients with high levels of LDL-cholesterol [8]. The mechanism is absorption of both food-derived cholesterol (400-500 mg/day) and bile acid-derived reabsorbed cholesterol (800-2000 mg/day). Generally, it is difficult to achieve the target LDL-cholesterol levels by dietary therapy alone in patients with high LDL-cholesterol. HMG-CoA reductase inhibitor, statin, is an inhibitor of cholesterol synthesis. Statin is the first-choice drug to reduce LDL-cholesterol for patients with high LDL-cholesterol, because of its strong effect to lower the circulating LDL-cholesterol levels [9]. Because a high dose of statins cause concern about rhabdomyolysis, it is sometimes difficult to achieve the guideline-recommended levels of LDL-cholesterol in patients with high LDL-cholesterol treated with statin monotherapy. Ezetimibe has been reported to reduce LDL-cholesterol safely with both monotherapy and combination therapy with statins [10]. Ezetimibe is especially expected to be effective in statin-intolerant patients with high LDL-cholesterol [11].

In previous studies, ezetimibe effectively and safety reduces blood LDL-cholesterol with both monotherapy and combination therapy [12-14]. Unfortunately, it is still unclear whether or not the LDL-cholesterol-lowering by ezetimibe is effective to suppress cardiovascular events or death [15]. Whether LDL-cholesterol lowering through dual inhibition of reduced cholesterol absorption and synthesis translates to enhanced clinical benefit for reducing coronary heart disease events awaits further assessment in longer-term, outcome-based clinical trials [16-18]. The results of these coronary heart disease outcome studies will help to better clarify the clinical importance of the metabolic effects of ezetimibe with respect to LDL-cholesterol lowering and beyond.

On the other hand, recent reports have shown that the ezetimibe therapy improves hypercholesterolemia-related metabolic disorders, such as postprandial hyperlipidemia in patients with hypercholesterolemia and non-alcoholic fatty

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liver disease (NAFLD) in high-fat fed mice [19, 20]. These data suggest that ezetimibe could have multifunctional atheroprotective effects in high-risk patients with hyperlipidemia and metabolic disorder, and could be the first-choice drug to improve entire coronary risk factors accumulated in patients with metabolic syndrome. Increasing oxidative stress in accumulated fat is an early instigator of metabolic syndrome and the redox state in adipose tissue is a potentially useful therapeutic target for obesity-associated metabolic syndrome [21]. Collectively, we hypothesized that the ezetimibe therapy improves excess ROS production-caused atherosclerosis in patients with hypercholesterolemia.

In this review, we propose a new therapeutic possibility of ezetimibe as a vascular protective drug fighting against oxidative stress resulting in atherosclerosis.

2. REACTIVE OXYGEN SPECIES IN VASCULAR SYSTEM

Oxidative stress plays an important role in the pathogenesis of cardiovascular disease, cancer, renal disease, and neurodegeneration [22]. ROS are a family of molecules including oxygen, such as superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$), and hypochlorous acid (HOCl). ROS contribute to vascular homeostasis at low levels as signaling molecules to mediate cell growth, migration, differentiation, and gene expression [23, 24]. In contrast, ROS at high levels induce cell death, apoptosis, and senescence. Therefore, excessive ROS production is commonly referred to as oxidative stress [25]. A large number of reports in the literature has linked oxidative stress with hypertension and atherosclerosis [26]. Vascular endothelial ROS released from a number of sources including the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase, myeloperoxidase, lipoxygenases, nitric oxide synthases (NOS), and the dysfunctional mitochondrial respiratory chain may play critical roles in ROS generation [4, 27, 28]. A multi-subunit NADPH oxidase is one of the major sources of ROS in vasculature and has been linked to hypertension and to pathological states associated with uncontrolled growth and inflammation, such as atherosclerosis [29]. In vascular ECs, NADPH oxidase consists of two transmembrane proteins (p22phox and gp91phox, also called NOX2, which together form the cytochrome b_{558}) and four cytosolic proteins (p47phox, p67phox, p40phox and a GTPase Rac1), which assemble at membrane sites upon cell activation [23, 30, 31] as shown in Fig. **1**. NADPH oxidase is activated by numerous stimuli including growth factors such as vascular endothelial growth factor (VEGF), angiopoietin-1, cytokines, shear stress, hypoxia and G-protein coupled receptor agonists including angiotensin II (Ang II) in ECs [29, 32, 33]. NADPH oxidase activates redox-sensitive genes (e.g. matrix metalloproteinase [MMP]; intercellular adhesion molecule [ICAM-1]; monocyte chemoattractant protein [MCP-1]; VEGF), which synthetically regulate in cell apoptosis, cell survival, inflammation, cell proliferation, hyperplasia, migration, and cell adhesion in vascular cells [28, 34]. NADPH oxidase-deficient apolipoprotein E (ApoE) knockout (ApoE (-/-)/p47phox (-/-)) mice had significantly less atherosclerosis compared with ApoE knockout mice [35]. Strong expression of NADPH oxidase subunit, Nox family proteins, is associated with increased ROS production and severity of atherosclerosis in human atherosclerosis [36]. ROS production from monocytes and macrophages, plate-

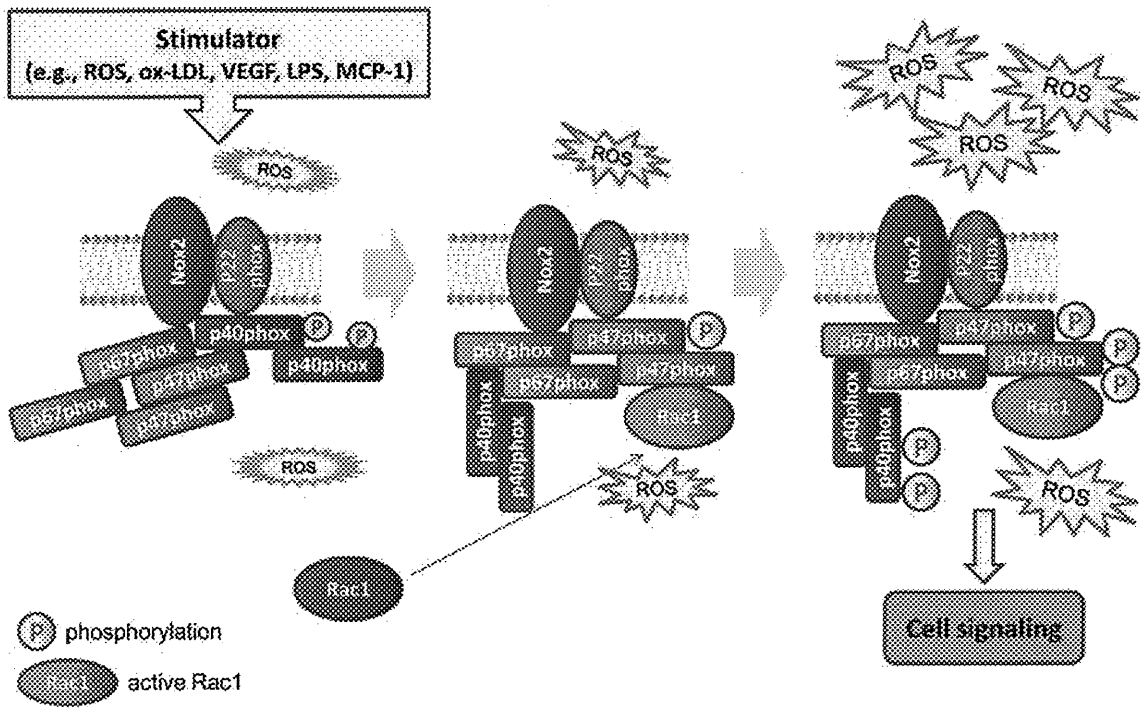

Fig. (1). Schematic diagram of the proposed concept of ROS generation in vascular endothelial cells (ECs). Nox2 (gp91phox) and its homologues (Nox1, Nox 4, and Nox5) and cytosolic components p57phox, p67phox, p40phox, and small GTPase Rac1 have been identified in ECs. ROS derived from endothelial NADPH oxidase function as signaling molecules.

lets, and vascular wall cells (e.g. ECs, smooth muscle cells) play a critical role in atherogenesis [37, 38]. During ROS-induced atherosclerosis, the activation of mitogenic signaling pathways in smooth muscle cells is also very important [37]. Antioxidants (e.g. superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase) could neutralize a certain amount of ROS, but excessive ROS productions surpass the scavenging capacity of cellular antioxidant systems. Therefore, the resulting oxidative stress leads to irreversible chemical changes in lipids (peroxidation), proteins and DNA (oxidation), consequently affecting cellular dysfunction and cytotoxicity [4]. As one of the endogenous atheroprotective mechanisms, a mechanosensitive group of antioxidant enzymes, the peroxiredoxin family, is upregulated by laminar shear stress in ECs [39].

Previous studies have shown an elevated extracellular release of ROS by mononuclear cells from hypertriglyceridemic and combined hyperlipidemic patients [40, 41]. Regarding the recruitment of circulating monocytes into the vascular intima with their subsequent transformation in foam cells, foam cell formation results from monocyte-derived macrophage scavenging of modified lipoproteins that have undergone oxidative modification in the vascular wall [42, 43]. The scavenger receptor and CD36 mediated oxidized LDL uptake by macrophages lead to the initial formation of the well-defined fatty streaks in the arterial intima. Such lesions then progress to more complex ones and are prone to rupture precipitating clinical events such as ischemic heart attack and stroke [43]. ROS production by peripheral blood monocytes are upregulated in patients with hyperlipidemia [8], which may contribute to systemic oxidative stress and atherosclerosis.

3. LIPID OXIDATION AND MOLECULAR SIGNALING

Altered cellular function resulting from protein, lipid, and DNA oxidation has been implicated in vascular inflammation. Remarkably, a large number of signaling pathways, including the mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K), protein kinase b (Akt), protein kinase C, and protein phosphatases are activated by ROS. In addition, ROS, such as peroxynitrite, hydrogen peroxide, and lipid peroxides, activate DNA binding factors (e.g. activator protein-1 [AP-1], nuclear factor-kappaB [NF-*k*B]) that, in turn, lead to transcription of several proinflammatory genes, including vascular cell adhesion molecule (VCAM)-1, ICAM-1, MCP-1, and E-selectin [44]. ROS also have been shown to activate MMPs and angiogenesis hallmarks of unstable atherosclerotic lesions [45]. Thus, ROS have been implicated in virtually every step in the atherosclerotic process [46]. Lipid oxidation is a particularly important aspect of the biochemistry of ROS in vascular system [47].

Oxysterols include oxygenated derivatives of cholesterol and plant sterols. Due to processing, heating or prolonged storage of animal-derived or deep-fried foods, the typical Western diet contains substantial quantities of oxidized cholesterol [48, 49]. Oxysterols are absorbed by the small intestine and incorporated into chylomicrons and LDL-cholesterol. Identified in the arterial wall, oxysterols may contribute to increased levels of oxidized lipoproteins in the intima [49].

LDL are susceptible to structural modifications by oxidation, particular, particularly the small dense LDL particles. The formation of lipid peroxidation derivates, such as thiobarbituric reactive substances, conjugated dienes, lipid hydroperoxides, and aldehydes, is associated with changes in apolipoprotein conformation and affects the functional properties of LDLs. The oxidized LDL (ox-LDL) is one of the most potent proatherosclerotic mediators compared to the native unmodified LDL [50, 51].

Ox-LDL receptors have been identified in the monocyte/macrophages, smooth muscle cells, and ECs. Among of all the receptors, the lectin-like receptor for ox-LDL (LOX-1) is predominantly expressed in ECs, and to a small extent in monocyte/macrophages, smooth muscle cells, and platelets. Use of a LOX-1-blocking antibody reduced almost all of the effects of ox-LDL on the EC biology described [52].

With regard to the immune mechanisms associated with the atherosclerotic process, it has been reported that ApoE knockout mice fed a standard chow diet synthesize exclusively T helper (Th)1-dependent immunoglobulin (IgG)2a anti-malondialdehyde (MDA)-modified LDL (anti-MDA-LDL), whereas antibody production is changed to Th2-dependent IgG-anti-MDA-LDL in severely hypercholesterolemic mice on a high-fat diet [53]. Therefore, dietary cholesterol administration induces a switch from Th1- to Th2-dependent autoimmune response. This could potentially be due to either a direct effect of the hypercholesterolemic state on the immune system or to a metabolic change caused by the high-fat diet, such as its content of cholic acid or different fatty acid composition [54]. To clarify the role of ROS-induced atherosclerosis, further studies including the immune response associated with hypercholesterolemia and atherosclerosis are warranted.

4. OXIDIZED LDL AS AN ATHEROGENIC CRIMINAL

Ox-LDL formation in subendothelial space of the arterial wall is a key initiating step in atherosclerosis, because it contributes foam cell generation, endothelial dysfunction, alteration of nitric oxide signaling, initiation of adhesion molecules expression in ECs that accelerate leukocyte homing to the site of atherosclerosis, and chronic vascular inflammation [55, 56]. One of the key observations that crystallized the important role of ox-LDL in atherosclerosis came from a study that showed heparin sulfate-dependent binding of ox-LDL to a subendothelial matrix [57]. Autoantibodies to ox-LDL have been found within normal/non-atherosclerosis and atherosclerosis-prone subjects. IgG autoantibodies to ox-LDL are associated with proatherogenic properties, and IgM autoantibodies to ox-LDL, including natural antibodies, have been proposed as atheroprotective properties [58]. Extensive atherosclerosis in ApoE knockout mice is associated with increased natural antibody titers to ox-LDL [58]. Most impressively, ox-LDL plays an integral role in the initiation of atherosclerosis (accumulation of inflammatory cells), to its progression (smooth muscle cell growth and proliferation), and the end event (plaque rupture and activation of platelets resulting in formation of a thrombus) [46]. In the past ten

years, immunoassays were developed using monoclonal antibodies against oxidation-dependent epitopes of LDL which made it possible to direct measurement of circulating ox-LDL. In a clinical study, 178 patients with coronary artery disease had a higher level of ox-LDL than 126 age-matched controls [59]. This finding has been corroborated in a large study comprising 1183 patients at high risk for coronary artery disease [60]. The odds ratio for high risk for coronary artery disease risk in the highest quartile of ox-LDL was 2.79, compared with the lower quintile and after adjusting for age, sex, race, LDL-cholesterol, smoking status, and C reactive protein (CRP) [60]. The Asklepios Study, investigating 2524 healthy middle-aged subjects, showed that circulating ox-LDL is affected by many biological and lifestyle factors, as well as generalized subclinical atherosclerosis [61]. Although it is still unclear whether ox-LDL elevations are a cause or a result of prevalent atherosclerosis [46], ox-LDL may be one of the useful biomarkers as a risk factor for the subsequent development of atherosclerosis in otherwise healthy subjects [62-66]. Specifically, circulating the ox-LDL level reflects early atherosclerotic changes and metabolic disorders including diabetes and obesity [67]. *In vitro* exposure to ox-LDL increased NF-*k*B activity in mononuclear cells, suggesting a pathogenic role of circulating ox-LDL in the exacerbation of oxidative stress [68]. The oxidative modification of LDL cholesterol to ox-LDL is thought to be a key initiating step in the development of atherosclerosis. Ox-LDL decreases the gene expression of the EC NO synthase (eNOS) and enhances ROS generation in ECs [69]. Moreover, ox-LDL also activates inflammatory cells and facilitates release of a large number of growth factors from monocytes and macrophages [70]. Later, ox-LDL acts as a persistent proinflammatory trigger for the progression of atherosclerosis and plaque rupture (atherothrombosis), responsible for the downstream clinical sequelae. Interestingly, ox-LDL is also a risk marker for early ventricular remodeling, independent of classic risk factors, lifestyle, inflammation, and prevalent vascular damage [61]. One mechanistic explanation for the observed effects on cardiac structure and function can be related to ox-LDL directly-induced myocardial damage [71] and intense contractile and electrophysiological changes [72]. Although the causality remains unproven, the inhibition of ox-LDL may be a useful therapeutic strategy to prevent non-ischemic cardiac failure in patients with atherosclerosis-prone.

5. EFFECTS OF EZETIMIBE IN LOW-DENSITY LIPOPROTEIN-CHOLESTEROL LOWERING AND BEYOND

Clinically, the ezetimibe therapy diminished circulating levels of total cholesterol and LDL-cholesterol in patients with primary hypercholesterolemia [73-75]. In monotherapy studies, ezetimibe generally decreased triglyceride (TG) levels compared with placebo [73, 76, 77]. Moreover, ezetimibe monotherapy has been reported to significantly decrease fasting levels of TG, apolipoproteinB (apoB)-48, and remnant lipoprotein cholesterol in patients with type ⅡB hypercholesterolemia [19]. When co-administered with statins, ezetimibe resulted in greater additional reductions in TGs levels compared with placebo [78-85]. The mechanism of ezetimibe-induced reduction of TG levels is still unclear. One of the mechanisms that ezetimibe improves lipoprotein profiles may be the suppression of intestinal chylomicrons (CM) production. Circulating TG are mainly found in TG-rich lipoproteins (TRL) consisting of CM and very low-density lipoproteins (VLDL). CM assemble dietary cholesterol, TG, and apoB-48 in enterocytes; and VLDL assemble endogenous hepatic TG, cholesterol, and apoB-100 in hepatocytes. In the study, the administration of ezetimibe improved endogenous and exogenous TRL profiles by suppressing postprandial intestinal production of CM and reducing the fasting hepatic cholesterol pool [19]. Thus, ezetimibe treatment could be a favorable therapeutic option for patients with elevated VLDL, LDL, and remnant lipoproteins, as in metabolic syndrome patients with hypercholesterolemia. Indeed, several studies have shown that ezetimibe improved lipid metabolism in obese patients with dyslipidemia and in animal models for metabolic syndrome [86-88]. In our clinical data, ezetimibe dramatically improved lipid profiles and a biomarker of oxidative stress in high-risk patients with hypercholesterolemia (Table **1**). The study was undertaken to investigate the effect of ezetimibe (10 mg/day) in combination with a statin on hypercholesterolemia in 12 high-risk patients of coronary artery disease. Ezetimibe add-on therapy induced a mean reduction in LDL-cholesterol of 27% ($p = 0.004$) at the 22nd week.

6. ATHEROPROTECTIVE MECHANISMS OF HIGH-DENSITY LIPOPROTEIN AND EZETIMIBE

Cholesterol efflux from peripheral tissues into plasma, then to the liver, in which cholesterol and its metabolic products (e.g. bile acids) are excreted into bile, is termed reverse cholesterol transport [89]. HDL-cholesterol mediates most reverse cholesterol transport and thus influences the amount of cellular components under normal and pathogenic conditions. HDL also has antioxidant, anti-inflammatory, anti-apoptotic, and vasodilatory properties. For instance, HDL inhibits expression of adhesion molecules and reduces leukocyte homing to arterial endothelium [90, 91]. Infusion of reconstituted HDL, suppression of cytokine, and chemokine production was observed in an inflammatory porcine model [92]. The mechanism of which may include that HDL-associated lysosphingolipids inhibit NADPH oxidase-dependent ROS generation and MCP-1 production in a process that requires coordinate signaling through sphingosine 1-phosphate (S1P)3 and scavenger receptor type B1(SR-B1) receptors [93].

The level of plasma HDL-cholesterol is inversely associated with the risk of developing atherosclerosis [94, 95], apparently in part because of its role in promising reverse cholesterol transport [96-99]. Study participants from the Framingham Study at the 80th percentile of HDL cholesterol were found to have half the risk of coronary heart disease developing when compared with subjects at the 20th percentile of HDL cholesterol [100]. One of the mechanisms of HDL-induced atheroprotective effect is the regulation of ROS-related vascular inflammations. HDL-associated lysosphingolipids inhibit NADPH oxidase-dependent MCP-1 [93]. Amazingly, HDL-cholesterol could lose their usual atheroprotective properties through specific chemical and structural alterations and could play a proinflammatory role by alteration of reverse cholesterol transport, enhanced oxi-

Table 1. Effect of Ezetimibe on Circulating Biomarkers in Patients with Hypercholesterolemia

Biomarkers	Ezetimibe (-)	Ezetimibe (+)	*P*-Value
Triglyceride (mg/dL)	205 ± 118	173 ± 66	0.355
LDL-cholesterol (mg/dL)	139 ± 16	102 ± 14	0.004
HDL-cholesterol (mg/dL)	55 ±7	56 ± 11	0.610
FFA	412 ± 250	378 ± 191	0.706
RLP-cholesterol (mg/dL)	11.2 ± 7.4	8.8 ± 5.4	0.187
Apolipoprotein B (mg/dL)	107 ± 16	90 ± 10	0.074
Apolipoprotein AI (mg/dL)	142 ± 12	147 ± 19	0.455
MDA-LDL (mg/dL)	112 ± 15	92 ± 29	0.222
Glucose (mg/dL)	123 ± 26	111 ±17	0.153
HbA1c (%)	6.1 ± 0.7	6.0 ± 0.8	0.529
hs-CRP (mg/dL)	0.10 ± 0.06	0.16 ± 0.20	0.407
dROMs (U Carr)	364 ± 46	335 ± 50	0.044

Data were shown as mean ± SD. The Student's paired *t*-test was used for pairwise comparisons between values before and after administration of ezetimibe for 22 weeeks (n = 12). LDL, low density lipoprotein; HDL, high density lipoprotein; FFA, free fatty acid; RLP, remnant lipoprotein; MDA, malondialdehyde-modified; HbA1c, haemoglobinA1c; hs-CRP, high sensitivity C-reactive protein; dROMs, derivatives of the reactive oxidative metabolites.

dation of LDL-cholesterol, and increased vascular inflammation linked to atherosclerosis [101].

In ApoE knockout mice, ezetimibe administration (5 mg/kg/day) for 3 months markedly reduced plasma levels of LDL-cholesterol (78% reduction) and increased HDL-cholesterol (87% increase) in high-fat diet mice [102]. Ezetimibe monotherapy elicits modest increases in HDL-cholesterol levels. In several ezetimibe monotherapy trials, 5 and 10 mg/day of ezetimibe provided additional increases in HDL-cholesterol from baseline levels compared with placebo (2.2% to 3.5%; $p < 0.05$ to $p < 0.01$) [73, 76, 77, 103]. The modest increase in HDL-cholesterol levels induced by ezetimibe may be attributed to the liver, which subsequently enhances the clearance of TRL. A reduction in the levels of TRL, which are acceptors of cholesterol transferred from HDL particles by cholesteryl ester transfer protein (CETP), may contribute to a reduced rate of cholesterol transfer, thereby increasing levels of HDL-cholesterol [104, 105]. In our clinical data, regarding patients with hyper LDL-cholesterolemia without low HDL-cholesterol, ezetimibe treatment had a tendency to increase serum HDL-cholesterol but not significantly (Table **1**).

To determine atheroprotective effects of ezetimibe, we evaluated ratios of proatherogemic to antiatherogenic lipoprotein measurements, LDL to HDL cholesterol (LDL/HDL cholesterol) and apolipoprotein B to apolipoprotein A-I (apolipoprotein B/A-I) [106]. As shown in Fig. **2**, ezetimibe improved the ratio of LDL/HDL cholesterol and apolipoprotein B/A-I. Most importantly, the most impressive data is that ezetimibe improved the ratio of LDL/HDL cholesterol (by 41%; from 2.7 to 1.6; $p < 0.001$) as shown in Fig. **2A**. According to a recent report in the literature, the lipid ratio of LDL/HDL is better monitoring predictors than single standard lipids including total cholesterol, LDL-cholesterol, and HDL-cholesterol [107]. Regarding initial risk measurements, several previous cohort studies and a meta-analysis study suggest that the ratios of LDL/HDL also have greater independent predictive values for coronary heart disease than individual serum total cholesterol or LDL-cholesterol levels [108-112]. In the current therapy for hypercholesterolemia, ezetimibe is the most powerful agent to improve the LDL/HDL ratio compared to any statin monotherapy.

Furthermore, ezetimibe add-on therapy significantly reduced the ratio of apolipoprotein B/A-I in high-risk patients with hypercholesterolemia as shown in Fig. **2B**. The add-on therapy with ezetimibe "significantly" reduced the apolipoprotein B/A-I ratio in high-risk patients with hypercholesterolemia (by 25%; from 0.8 to 0.6; $p = 0.029$). Patients who were treated adequately according to current guidelines (i.e. LDL cholesterol $\leq$ 100 mg/dl) still had residual major cardiovascular event risks that could be recognized by the

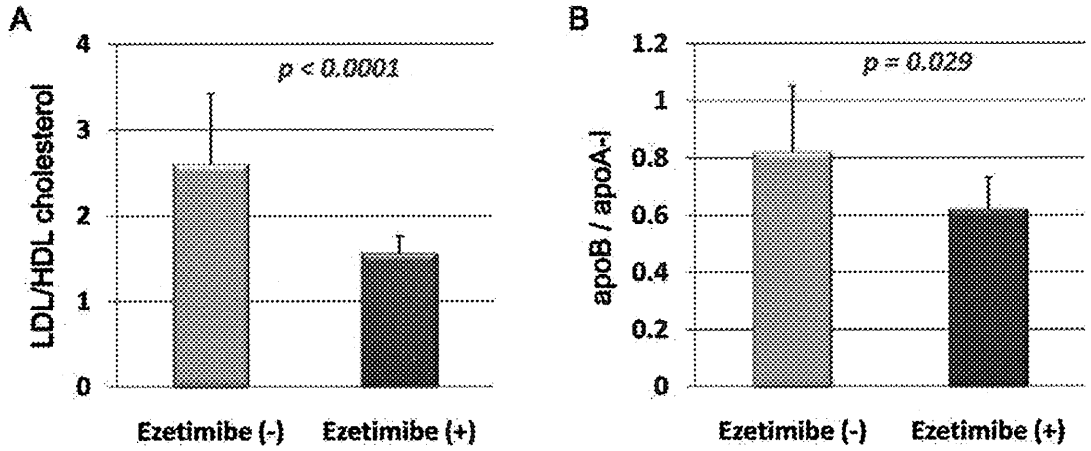

Fig. (2). Ezetimibe improved lipid profiles in patients with hypercholesterolemia. A, Ezetimibe improved LDL/HDL- cholesterol ratio in patients with hypercholesterolemia. B, Ezetimibe improved apolipoproteinB (apoB) to apolipoproteinA-I (apoA-I) ratio in patients with hypercholesterolemia. LDL-cholesterol, HDL-cholesterol, apoB, and apoA-1 were measured before and after 22-week ezetimibe administration in patients with athrosclerosis-prone hypercholesterolemia (n = 12; mean age, 63 ± 3 years; 50% women). Bars represent the mean value of the ratio ±SD.

evaluation of levels of non-HDL cholesterol (the sum of the cholesterol concentration in all proatherogenic lipoproteins [VLDL, IDL, and LDL particles]) or apolipoprotein B (the major apolipoprotein of these particles) [110]. On-treatment levels of non-HDL cholesterol and apolipoprotein B are considered to be more closely associated with cardiovascular outcome than levels of LDL cholesterol [113]. These data suggest that ezetimibe not only reduce serum levels of LDL cholesterol but also improve total lipid profiles.

7. MOLECULAR TARGET OF EZETIMIBE AS AN ATHEROPROTECTIVE DRUG

Ezetimibe, a novel lipid-lowering agent, selectively inhibits intestinal cholesterol absorption, reducing total cholesterol and triglyceride levels and also reducing the development of atherosclerosis in ApoE knockout mice [114, 115]. According to data from apoE and eNOS double knockout mice, lipid lowering with ezetimibe potentially reduced atherosclerosis and vascular inflammation independent of eNOS [116]. A sterol transporter, Niemann-Pick C1-Like 1 (NPC1L1) is involved in subcellular cholesterol trafficking and plays a critical role in the absorption of intestinal cholesterol [117, 118]. NPC1L1-deficient mice exhibit a substantial reduction in absorbed cholesterol, on which ezetimibe had no effect [118]. Thereafter, the molecular target of ezetimibe was revealed to be NPC1L1, which is a critical mediator of cholesterol absorption and an essential component of ezetimibe-sensitive pathway [115]. Ezetimibe was reported to bind specifically to a single site in mammalian enterocyte brush border membranes and to human embryonic kidney cells expressing NPC1L1 [115]. In an individual with dyslipidemia who did not respond to treatment with ezetimibe, compound heterozygosity for 2 rare polymorphisms of the NPC1L1 gene was identified [119].

NPC1L1 is widely expressed in many human tissues, with the highest expression in the small intestine as well as in the liver [120, 121]. Therefore, ezetimibe inhibits cholesterol absorption in the small intestine, reduces enterohepatic circulation of cholesterol, reduces uptake of free cholesterol in hepatocytes, and should affect metabolic pathways in the liver [122]. Ezetimibe improved hepatic insulin signaling as well as hepatic steatosis in Zucker Obese fatty (ZOF) rats. Ezetimibe also restored insulin sensitivity in steatotic hepatocytes *in vitro* by a reduction in hepatic ROS generation, Janus-family tyrosine kinase (JNK) activation, and endoplasmic reticulum stress. In addition, ezetimibe recovered insulin-induced Akt activation, and reduced gluconeogenic genesin the liver of ZOF rats and cultured steatotic hepatocytes [122]. Other studies have shown that ROS-dependent activation of JNK plays a role in the developing insulin resistance [123-125]. In recent clinical studies, ezetimibe treatment has been considered as an effective therapeutic option for NAFLD [120, 126, 127]. Several reports have concluded that ezetimibe monotherapy not only protects against high fat diet-induced dyslipidemia but also attenuates liver steatosis in an experimental NAFLD model [88, 128]. In ApoE knockout mice, liver weight was significantly decreased and lipid accumulation in the liver was also dramatically inhibited in the ezetimibe-treated group [102]. These accumulating data suggest that the inhibition of NPC1L1-dependent cholesterol uptake by ezetimibe may be a suitable therapeutic target for treatment of not only hypercholesterolemia but also broader aspects of metabolic disorders in patients with type 2 diabetes and/or metabolic syndrome as shown in Fig. 3.

8. BIOMARKERS FOR OXIDATIVE STRESS AND EZETIMIBE

With regard to ezetimibe, several clinical studies have already revealed that ezetimibe therapy improves biomarkers for oxidative stress [129, 130]. Furthermore, ezetimibe decreased serum asymmetric dimethylarginine (ADMA) levels, urinary excretion levels of 8-hydroxydeoxyguanosine (8-OHdG), markers of oxidative stress, and proteinuria in pa-

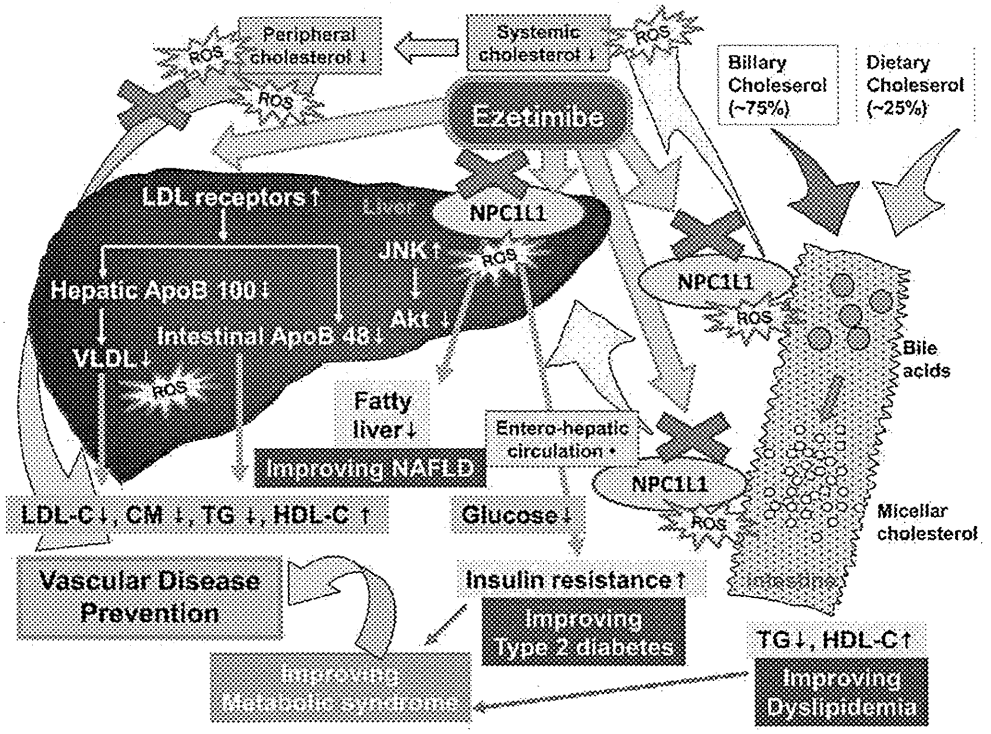

Fig. (3). Schematic diagram of the proposed mechanisms of ezetimibe-induced atheroprotective effects through reactive oxygen species (ROS)-related pathways. LDL-C: low-density lipoprotein-cholesterol; ox-LDL: oxidized LDL; VEGF: vascular endothelial growth factor; LPS: lipopolysaccharide; MCP-1: monocyte chemotactic protein-1; CM: chylomicrons; TG: triglyceride; HDL-C: high-density lipoprotein-cholesterol; NAFLD: non-alcoholic fatty liver disease; NPC1L1: Nieman-Pick C1-like 1; apoB: apolipoproteinB; VLDL: very low-density lipoprotein; JNK: Janus-family tyrosine kinase.

tients with non-diabetic chronic kidney disease with dyslipidemia [130].

Based on the site of ROS release and their activity, a large number of biomarkers of oxidative stress have been proposed, such as ox-LDL, antibodies to ox-LDL, F2-isoprostanes, thiobarbituric acid reactive substances (TBARS), free oxygen radical monitor (FORM) (derivatives of reactive oxidative metabolites, dROMs), 8-OHdG [46, 131-135].

Of all others, the FORM assay detecting organic peroxides known as the dROMs test was available [136, 137]. This assay is relatively inexpensive, simple, easy, and can be performed in 5 minutes. In previous reports, the dROMs assay was used to assess the effectiveness of various antioxidant treatment strategies [137, 138]. To examine whether ezetimibe improves oxidative stress or not, we studied ezetimibe add-on therapy with a statin. The ezetimibe add-on therapy remarkably reduced dROMs levels in high-risk patients with hypercholesterolemia as shown in Fig. **4**. Considering that ezetimibe monotherapy or a combination therapy with simvastatin decreased LDL tendency to peroxidation, ezetimibe may have favorable pleiotropic effects beyond the LDL-cholesterol lowering [129, 130]. These data suggest that ezetimibe could contribute to atheroprotective properties through effective anti-oxidant actions. Since ox-LDL induces expression of a NADPH oxidase, Nox2, and ROS generation in vascular ECs [139], ezetimibe may attenuate ROS production through reduction of circulating ox-LDL in patients with hypercholesterolemia.

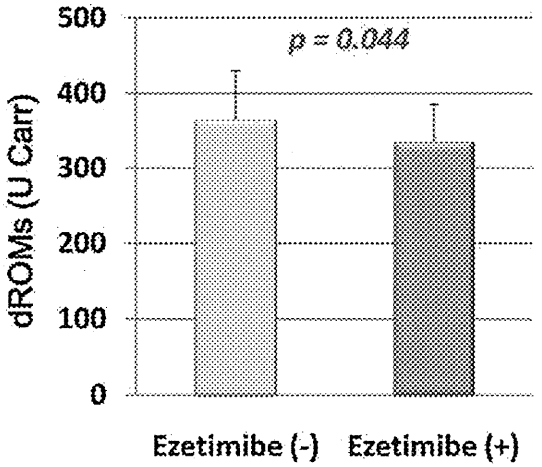

Fig. (4). Ezetimibe reduced a circulating oxidative marker, derivatives of reactive oxidative metabolites (dROMs), in patients with atherosclerosis-prone with hypercholesterolemia. Measurements of dROMs were assessed before and after 22-week ezetimibe administration in patients with athrosclerosis-prone hypercholesterolemia (n = 12; mean age, 63 ± 3 years; 50% women). Bars represent the mean value of the ratio ±SD.

9. EFFECTS OF EZETIMIBE TO IMPROVE ENDOTHELIAL FUNCTION IN PATIENTS WITH DYSLIPIDEMIA

Endothelial dysfunction is the initial step of atherosclerosis. A recent report demonstrated that ezetimibe treatment attenuated vascular functions such as endothelial dysfunction, oxidative stress, and inflammation in high-fat fed ApoE-deficient mice [102]. Ezetimibe treatment markedly inhibited the development of lipid-rich plaque and also significantly improved endothelial dysfunction assessed by the vasodilator response to acetylcholine [140], accompanied by inhibition of interleukin-6 mRNA and an increase in eNOS mRNA in the aorta. Furthermore, ezetimibe suppressed ROS generation and the ubiquitination-proteasome system in the aorta.

In our clinical data, after 22 weeks of ezetimibe add-on combination therapy with a statin, reactive hyperemia index (RHI) by the EndoPAT™, a fingertip peripheral arterial tonometry device [141, 142], was improved in high-risk patients with hypercholesterolemia as shown in Fig. **5**. According to a previous study, an RHI < 1.35 was found to have a sensitivity of 80% and a specificity of 85% to identify patients with coronary endothelial dysfunction [143]. Ezetimibe add-on therapy achieved effective RHI-improvement (mean RHI 1.32 to 1.53, p = 0.020) in high-risk patients with hypercholesterolemia.

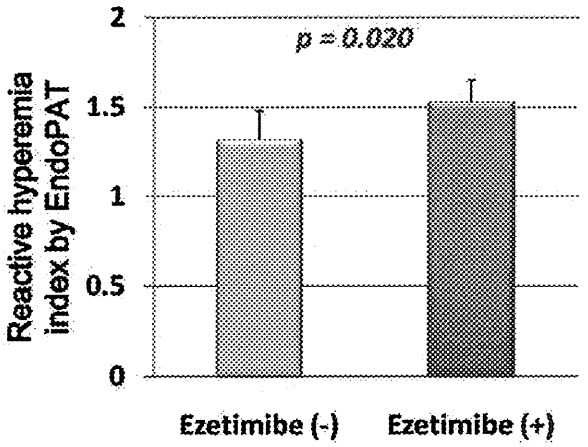

Fig. (5). Ezetimibe improved reactive hyperemia index (RHI) by EndoPAT™ in patients with hypercholesterolemia. Using a fingertip peripheral arterial tonometry device, we measured digital pulse amplitude in patients with athrosclerosis-prone hypercholesterolemia (n = 12; mean age, 63 ± 3 years; 50% women) for 5 minutes at baseline and after a reactive hyperemia induced by 5-minute forearm cuff occlusion. RHI were measured before and after 22-week ezetimibe administration in patients with hypercholesterolemia. Bars represent the mean value of the ratio ±SD.

Considering mechanisms of improving endothelial function, cholesterol lowering may be more important than pleiotropic effects of lipid-lowering drugs for improvement in endothelial function in patients with dyslipidemia and coronary artery disease [144].

Through the inhibition of chemokine (C-C motif) receptor 2 (CCR)2-mediated recruitment of monocytes into the vessel wall by activating intracellular signaling pathways, ox-LDL activates peroxisome proliferator-activated receptor gamma (PPARγ) [145]. However, endothelial PPARγ regulates vascular NO production and contribute to endothelial dysfunction [146]. Activation of PPARγ attenuated both metabolic syndrome and atherosclerosis in aging mice through a novel antioxidant mechanism (DJ-1 and forkhead box, subgroup O family, FOXOs; nuclear factor [erythroid-derived 2]-like 2, Nef2) to enhance vascular antioxidant responses in lipoprotein receptor knockout mice [147]. Ezetimibe-affected vascular endothelial function may be attributed not only to its blocking of cholesterol absorption but also to decreased ROS-mediated NO inactivation and enhanced antioxidant systems.

CONCLUSIONS

In conclusion, ezetimibe inhibits NPC1L1 controlling cholesterol absorption in the intestine, reduces excessive ROS production, and prevents atherosclerosis in vasculature. Moreover, ezetimibe improves NPC1L1 contributing hepatic insulin resistance and fatty liver through suppressing free cholesterol accumulation and subsequent ROS generation. As an atheroprotective drug, ezetimibe may be a suitable therapeutic target for treatment of not only hypercholesterolemia but also broader aspects of metabolic disorders in patients with type 2 diabetes and/or metabolic syndrome.

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LIST OF ABBREVIATIONS

8-OHdG	=	8-hydroxydeoxyguanosine
ADMA	=	Asymmetric dimethylarginine
Akt	=	Protein kinase b
Ang II	=	Angiotensin II
AP-1	=	Activator protein-1
apoA-1	=	Apolipoprotein A-1
apoB	=	ApolipoproteinB
apoE	=	Apolipoprotein E
CCR	=	Chemokine (C-C motif) receptor
CETP	=	Cholesteryl ester transfer protein
CM	=	Chylomicrons
CRP	=	C reactive protein
DJ-1	=	Parkinson disease 7 (PARK7)
dROMs	=	Derivatives of reactive oxidative metabolites
eNOS	=	Endothelial cell NO synthase
ECs	=	Endothelial cells
FORM	=	Free oxygen radical monitor