3 ガイドライン

糖尿病を合併する高血圧患者においては，血圧をより低く下げた方が，大血管障害と細小血管障害のリスクを著明に減少させる(糖尿病合併症全体が24％，細小血管症が37％，脳血管障害が44％，心筋梗塞が21％)というUKPDS(United Kingdom Prospective Diabetes Study)39の成績[3]や，降圧目標が拡張期血圧80 mmHg未満の群の心血管疾患発症率は，90 mmHg未満を目標とした群に比べ，約50％低下したというHOT(Hypertension Optimal Treatment) studyの成績[4]からは，糖尿病を合併した高血圧に対し，目標血圧値をより低く設定することが大きな治療効果をもたらすと考えられる．これらの結果からJNC-VIやWHO/ISHガイドライン，JSH2000では，130/85 mmHg以上の正常高値血圧から治療対象となっていた．その後のエビデンスの追加により，降圧目標をより低い130/80 mmHg未満に設定するようになってきた．我が国でも端野・壮瞥町研究において，糖代謝異常のない群では収縮期血圧が140 mmHg以上，拡張期血圧が90 mmHg以上から心血管疾患死亡に対する相対危険度の上昇を認めたのに対し，糖代謝異常(境界型糖尿病および糖尿病)群では，収縮期血圧130 mmHg以上，拡張期血圧80 mmHg以上から相対危険度の上昇を認めた．更に，糖代謝異常群では，120/80 mmHg未満の至適血圧群に比べて心血管疾患による死亡率が有意に増加しており，降圧目標を130/80 mmHg未満とすることを指示している[5]．最近の主なガイドラインの概要を**表3**に示すが，JSH2009と同様に降圧目標は，130/80 mmHg未満に設定されている．補足として，ヨーロッパ高血圧学会(ESH)は，ESH/ESC2007の再評価という形で，すべての高血圧患者における降圧は，130-139/80-89 mmHgを目指し，できればその下限値である130/80 mmHgに近づけるとした．ただし，糖尿病患者では，降圧目標値は明示せず，できるだけ降圧する，との表現にとどまった．また，米国糖尿病学会(ADA)も，2011年のClinical Practice Recommendationの中で，収縮期血圧はほとんどの患者で130 mmHg未満を目指すが個々の病態に応じて決定し，拡張期血圧は80 mmHg未満とすると，降圧目標値のニュアンスを改訂している．

2010年に報告されたACCORD(Action to Control Cardiovascular Risk in Diabetes)血圧試験では，収縮期血圧120 mmHg未満の厳格管理群と，140 mmHg未満の標準管理群を比べると，一次エンドポイント(非致死性心筋梗塞・非致死性脳卒中・心血管疾患による死亡)の年間発生率は，厳格管理群1.87％，標準管理群2.09％とハザード比0.88で有意差に至らなかった．また，脳卒中に関してはハザード比0.59と厳格管理群で有意に低下していた[6]．したがって，現在，降圧目標値としている130/80 mmHg未満という数値もエビデンスが十分というわけではなく，今後，我が国の日本人でのエビデンスを構築することが重要であることを理解しておく必要がある．

4 治療指針

JSH2009に従って，糖尿病を合併した高血圧患者は，130/80 mmHg以上で治療を開始する．ただし，血圧が130-139/80-89 mmHgの場合，生活習慣の改善によって降圧目標達成が見込める場合は，3カ月以内の範囲で生活習慣の改善による降圧を試みてもよいとされている．従来は，3-6カ月とされていたので，大きく変更されている．塩分制限を中心とした食事療法や有酸素運動の降圧効果は確立されている．食塩6 g/日未満の食塩摂取量および野菜，果物，魚，低脂肪乳製品などの摂取が高血圧患者にとって降圧効果をもたらし，中等度強度の有酸素運動が，血圧低下のみならず，体重，体脂肪，腹囲の減少や，インスリン感受性，HDLコレステロールの改善をもたらすことが示されている．したがって，まずは可能なかぎり食事療法，運動療法を中心とした生活習慣の改善が重要であることは言うまでもない．生活習慣の改善が困難な場合や降圧目標値に到達しない場合は，降圧薬の投与を検討する．

表3 糖尿病を伴う高血圧に関する各種ガイドライン
（片山茂裕：糖尿病に合併した高血圧，動脈硬化予防 10(1)：70-75，2011.より引用）

	JSH2009	ESH/ESC2007	ADA2004	JNC7(2003)
降圧目標	＜130/80 mmHg	＜130/80 mmHg	＜130/80 mmHg	＜130/80 mmHg
第一選択薬	ACEIかARB	すべてのクラスの降圧薬が可．ほとんどの例で2つ以上の降圧薬が必要．単剤ならRA系抑制薬を，2剤以上の併用でも1剤はRA系抑制薬を．	ACEI，ARB，D，βB，CCB．通常2つ以上の降圧薬が必要．1剤はRA系抑制薬を．	サイアザイド系D，βB，ACEI，ARB，CCB．通常2つ以上の降圧薬が必要．
注	単剤で効果不十分ならばCCBかD．3剤必要ならばRA系抑制薬＋CCB＋D．労作性狭心症・陳旧性心筋梗塞があればβB．	微量アルブミン尿がある場合には，正常高値血圧でもRA系抑制薬を．	目標血圧達成にサイアザイド系Dも併用を．RA系抑制薬の使用時には腎機能に注意．1型ではACEI，2型で微量アルブミン尿ではACEIかARBを，タンパク尿ではARBを．	ACEIやARBは腎症の進展を遅らせ，微量アルブミン尿を減少させる．また，ARBはタンパク尿への進展を遅らせる．

ACEI：ACE阻害薬，ARB：アンジオテンシンII受容体拮抗薬，βB：β遮断薬，CCB：Ca拮抗薬，D：利尿薬，RA系抑制薬：レニン・アンジオテンシン系抑制薬．

表3に示した4つのガイドラインにおいて，第一選択薬はレニン・アンジオテンシン系抑制薬であるACE阻害薬またはアンジオテンシンII受容体拮抗薬(ARB)が含まれている．MICRO-HOPE(Heart Outcomes Prevention Evaluation) studyでは，高血圧患者56％を含むハイリスク糖尿病患者を対象として，ACE阻害薬がプラセボ群に比較して心血管疾患発症率および死亡を25％減少させている[7]．また，LIFE(Losartan Intervention for Endpoint Reduction in Hypertension) studyでは，糖尿病患者を対象として，ARBであるロサルタンが，β受容体遮断薬に比べて，心血管疾患による死亡率を37％減少させ，全死亡を39％減少させている[8]．

JSH2009でも，糖尿病を合併した高血圧患者に対しては，ACE阻害薬あるいはARBを第一選択薬とし，効果不十分な場合は，増量するか，長時間作用型カルシウム拮抗薬あるいは少量のサイアザイド系利尿薬を第二選択薬としている．更に，降圧を要する場合には，3剤を併用する．ACE阻害薬，ARB，長時間作用型のジヒドロピリジン系カルシウム拮抗薬は，脂質代謝に影響を及ぼさず，インスリン感受性を改善すると報告されているが，糖尿病新規発症抑制効果は，ACE阻害薬とARBの方がカルシウム拮抗薬より強いことより，インスリン抵抗性改善効果が強いことが示されている[9]．ACE阻害薬は，非高血圧患者でもタンパク尿を伴う1型糖尿病患者における腎機能低下を抑制し，透析療法移行を減少させることが報告されており，我が国のJ-MIND[10]では，カルシウム拮抗薬とACE阻害薬が糖尿病性腎症のタンパク尿や腎機能に対して同等の効果があることも明らかにされている．また，SMART[11]では，ARBの糖尿病性腎症に対する有用性が明らかにされた．

JSH2009では，尿タンパク1g/日以上の糖尿病性腎症を伴った高血圧患者では，特に降圧を厳格にすべきで，125/75 mmHgを降圧目標としている．糖尿病性腎症におけるレニン・アンジオテンシン系抑制薬との併用薬として，カルシウム拮抗薬と利尿薬を比較したGUARD[12]では，タンパク尿減少には利尿薬，eGFR保持にはカルシウム拮抗薬の併用で効果が強いことが

示されている．β受容体遮断薬に関しては，糖尿病患者に生じる低血糖症状を自覚しにくくする作用があり，注意が必要ではあるが，心保護作用を有しているので必要に応じて血圧管理に使用する．

5 今後の課題

UKPDSは，新たに診断された2型糖尿病患者が対象であったが，血圧の厳格な管理が血糖管理と同等の有効性を認めた．厳格な血糖管理は，血圧管理に比べて心血管疾患の発症をあまり減少させなかったが，その一定期間の厳格な血糖管理が，その後通常の治療になっても長期間効果が残る，いわゆる'legacy effect'が注目されている．それに対し，厳格な血圧管理における細小血管症や大血管障害低減効果は，年月の経過とともに明らかでなくなったことが報告された[13]．糖尿病患者の血圧管理はその後，一生涯継続する必要があることが示唆される．

おわりに

糖尿病合併症発症・進展阻止のための血圧管理は，130/80 mmHg未満を目指して徹底した降圧治療の継続が重要であり，腎症の発症予防，進展阻止のためにACE阻害薬あるいはARBが第一選択薬となる．降圧目標値に到達できない場合は，カルシウム拮抗薬または少量のサイアザイド系利尿薬を適宜追加，併用すべきである．ただし，治療開始時期や降圧目標に関する日本人のエビデンスは確立していないため，現在進行中の臨床試験の結果を参考に再検討されるであろう．

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Original article

Effect of cardiac rehabilitation on muscle mass, muscle strength, and exercise tolerance in diabetic patients after coronary artery bypass grafting

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ABSTRACT

Background: The effects of cardiac rehabilitation (CR) on muscle mass, muscle strength, and exercise tolerance in patients with diabetes mellitus (DM) who received CR after coronary artery bypass grafting (CABG) have not been fully elucidated.
Methods: We enrolled 78 consecutive patients who completed a supervised CR for 6 months after CABG (DM group, $n=37$; non-DM group, $n=41$). We measured mid-upper arm muscle area (MAMA), handgrip power (HGP), muscle strength of the knee extensor (Ext) and flexor (Flex), and exercise tolerance at the beginning and end of CR.
Results: No significant differences in confounding factors, including age, gender, ejection fraction, or number of CR sessions, were observed between the two groups. At the beginning of CR, the levels of Ext muscle strength and peak VO_2 were significantly lower in the DM group than in the non-DM group. At the end of CR, significant improvement in the levels of muscle strength, HGP, and exercise tolerance was observed in both groups. However, the levels of Ext muscle strength, HGP, peak VO_2, thigh circumference, and MAMA were significantly lower in the DM group than in the non-DM group. In addition, no significant improvement in thigh circumference and MAMA was observed in the DM group. At the end of CR, the levels of thigh circumference and MAMA correlated with Ext and Flex muscle strength as well as with HGP. Percent changes in the levels of Ext muscle strength were significantly correlated with those of MAMA and hemoglobin A1c.
Conclusions: These data suggest that improvement in muscle strength may be influenced by changes in muscle mass and high glucose levels in DM patients undergoing CR after CABG. A CR program, including muscle mass intervention and blood glucose control, may improve deterioration in exercise tolerance in DM patients after CABG.

Introduction

Patients with diabetes mellitus (DM) are at increased risk of coronary artery disease (CAD) [1]. Patients with DM are at 2–4 times higher risk of developing CAD and mortality due to CAD compared with non-DM patients [2]. Patients with CAD are treated by lifestyle modification, medical therapy, and coronary revascularization such as percutaneous coronary intervention and coronary artery bypass grafting (CABG). However, the benefits of revascularization are less and the risks and complications are greater than those in non-DM patients. Previous studies have also reported a high incidence of bypass graft dysfunction and a high mortality even in DM patients who underwent CABG [3].

Cardiac rehabilitation (CR) has numerous benefits such as modification of risk factors and prevention of future cardiovascular events [4]. Improvement in peak VO_2 after CR reduced cardiovascular morbidity and mortality in patients with CAD [5]. However, a previous study demonstrated that the presence of DM was a negative factor for improvement in peak VO_2 [6]. Another report showed a significant inverse relationship between fasting blood glucose

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levels and changes in peak VO_2 in CR participants with DM after coronary events [7]. Park et al. reported that a low level of muscle strength was a predictor of physical limitation, and diabetes was associated with a low level of skeletal muscle strength and deterioration in quality [8]. We recently reported that muscle strength and exercise tolerance were significantly lower in DM patients than non-DM patients at the beginning of CR after CABG [9]. However, the effects of CR on muscle mass, muscle strength, and exercise tolerance in DM patients undergoing CR after CABG has not been fully elucidated. The aim of this study was to investigate the effects of CR on muscle mass, muscle strength, and exercise tolerance in DM patients who received CR after CABG.

Methods

Subjects

We enrolled 78 consecutive patients who completed a supervised CR for 6 months after CABG at Juntendo University Hospital from July 2002 to February 2005. The patients were divided into 2 groups: those with DM (DM group, $n=37$) and those without DM (non-DM group, $n=41$), according to the guidelines of the Japan Diabetes Society (JDS), which includes history of medical treatment, fasting plasma glucose $\geq$ 126 mg/dl or casual plasma glucose $\geq$ 200 mg/dl, and hemoglobin (Hb) A1c $\geq$ 6.1% [10]. All patients participated in the CR program 6–8 days after CABG. All subjects gave written informed consent and the ethical committee of the institution approved this study.

Rehabilitation protocol

The CR program consisting of warm-up stretching, aerobic exercise, resistance training, and cool-down, was scheduled once or twice a week for 6 months, as described previously [11,12]. Aerobic exercise consisted of a cycle ergometer, treadmill, and walking on an indoor track with a total duration of approximately 60 min exercise intensity was prescribed individually at the anaerobic threshold (AT) level, as measured by an ergometer test using expiratory gas analysis or a rating of 11–13 on a standard Borg's perceived exertion scale. Resistance training, which was gradually added to the exercise program at least 6 weeks after participation, included sit-ups, back kicks and front raises, squats, and push-ups, using the patient's own weight. This training consisted of 1–2 sets of 10–15 repetitions for each muscle group with 3–5 min rest between sets. Patients were encouraged to perform home-based aerobic exercise twice a week for more than 20 min at a rating of 11–13 of perceived exertion on Borg's scale. All subjects were instructed to follow the phase II diet of the American Heart Association at the beginning of CR. An educational program regarding CAD and its risk factors at baseline was also provided for each subject by physicians, nurses, and dietitians.

Measurements

We assessed body composition, muscle strength, and exercise tolerance at the beginning and end of CR. Anthropometric parameters were assessed using body mass index (BMI), waist size, thigh circumference, triceps skin fold thickness measured on the dominant hand, and mid-upper arm circumference. Thigh circumference was measured directly below the gluteal fold of the right thigh according to WHO standards [13]. Mid-upper arm muscle area (MAMA) was calculated according to a standard method [14]. The percentages of body fat and lean body weight were measured by BOD POD® (Life Measurement, Inc., Concord, CA, USA), as described previously [11,12]. The power of the thigh muscles was measured using the Cybex770 system (Cybex Division of Lumex, Ronkonkoma, NY, USA), as also reported previously [11,12]. The isokinetic peak torques of the knee extensor (Ext) and flexor (Flex) muscles were measured at 60°/s, and those were adjusted by body weight according to the following formula: strength (Nm) × 100/kg body weight. Handgrip power (HGP) in the dominant hand was also measured. To measure peak oxygen consumption (peak VO_2) and oxygen uptake at the AT, patients underwent ergometer testing (Corival 400, Lobe B.V., Groningen, Netherlands) using an expiratory gas analysis machine (Vmax-295, SensorMedics Co., Yorba Linda, CA, USA). After a period of resting, warm-up was performed for a few minutes at 20 W, followed by ramp loading (15 W/min) until subjective exhaustion, progressive angina, ST-segment depression ($\geq$2 mm), or sustained tachyarrhythmia. The point of AT was determined by the "V-slope" method.

Statistical analyses

The results are expressed as mean ± standard deviation and were analyzed using the StatView software (Version 5.0J for Windows, SAS Institute, Cary, NC, USA). Comparisons between the DM and non-DM groups were performed by Student's *t*-test. Data at baseline and after 6 months of CR were compared for each patient by paired *t*-test to evaluate the singular effects of CR. Correlation coefficients were determined by linear regression analysis. Statistical significance of correlation coefficients was determined by the method of Fisher and Yates. A *p*-value of less than 0.05 was considered significant.

Results

Characteristics of CR subjects

The clinical characteristics of the subjects are presented in Table 1. Thirty-seven patients were diagnosed as having DM. No significant differences with regard to age, gender, coronary risk factors, number of diseased vessels, ejection fraction, or physiological variables, were observed between the DM and non-DM groups. Thirty-six patients received complete revascularization using the off-pump operation. One patient who had received re-CABG was in the DM group. No significant differences in the concomitant use of drugs, including antiplatelets, calcium channel blockers, β-blockers, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, or statins, were observed between the two groups. In the DM group, 24 patients (65%) and 13 patients (35%)

Table 1
Clinical characteristics of the study subjects.

	DM	Non-DM	*p*-Value
N	37	41	
Age (year)	63.5 ± 10	64.1 ± 9	NS
Male (%)	29 (78)	39 (95)	NS
Hypertension (%)	22 (61)	30 (73)	NS
Dyslipidemia (%)	28 (76)	31 (76)	NS
Current smoker (%)	17 (49)	21 (53)	NS
Familial history (%)	11 (26)	9 (26)	NS
History of MI (%)	2 (5)	0 (0)	NS
History of PCI (%)	2 (5)	0 (0)	NS
History of previous CABG (%)	1 (3)	0 (0)	NS
Diseased vessels			
LMT (%)	9 (25)	2 (5)	NS
3VD (%)	18 (48)	28 (68)	NS
1–2VD (%)	10 (27)	11 (27)	NS
Ejection fraction (%)	59.7 ± 16	65.3 ± 12	NS
Off-pump CABG (%)	36 (97)	41 (100)	NS
Exercise in hospital (times)	16 ± 14	18 ± 14	NS

Data are presented as the mean value ± SD. DM, diabetes mellitus; MI, myocardial infarction; PCI, percutaneous coronary intervention; CABG, coronary arterial bypass grafting; LMT, left main trunk; VD, vessel disease.

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were treated with oral anti-diabetic agents and insulin, respectively. No significant differences were observed between the two groups in exercise duration in the CR program (data not shown). No subject in either group showed any worsening of symptoms or had cardiovascular events during the 6 months of the study.

Serum lipid profiles and glucose parameters

Serum lipid profiles and glucose parameters at baseline and the end of CR are presented in Table 2. Fasting blood glucose and HbA1c levels before and after CR were significantly higher in the DM group than in the non-DM group (both $p < 0.05$). Lipid profiles were not significantly different between the two groups at both baseline and the end of CR.

Anthropometric parameters

The anthropometric parameters at baseline and after CR in both groups are presented in Table 3. The anthropometric parameters at baseline were not significantly different between the two groups. In the non-DM group, waist circumference (from 84.5 ± 7.8 to 82.2 ± 6.7 cm, $p < 0.05$) was significantly decreased, and thigh circumference (from 48.9 ± 4.1 to 50.7 ± 3.7 cm, $p < 0.05$), arm forced circumference (from 29.0 ± 2.6 to 30.0 ± 2.4 cm, $p < 0.05$), mid-upper arm muscle circumference (from 25.7 ± 2.5 to 26.5 ± 2.4 cm, $p < 0.05$), and MAMA (from 53.2 ± 10.3 to 56.5 ± 10.0 cm^2, $p < 0.05$) were significantly increased. In the DM group, waist circumference (from 83.4 ± 8.3 to 80.2 ± 5.7 cm, $p < 0.05$) was significantly decreased, however, thigh circumference, arm forced circumference, mid-upper arm muscle circumference, and MAMA were not significantly altered. At the end of CR, thigh circumference (47.3 ± 2.5 cm vs. 50.7 ± 3.7 cm, $p < 0.05$), arm forced circumference, (28.4 ± 1.6 cm vs. 30.0 ± 2.4 cm, $p < 0.05$), mid-upper arm muscle circumference (25.0 ± 1.8 cm vs. 26.5 ± 2.4 cm, $p < 0.05$), and MAMA (49.9 ± 7.1 cm^2 vs. 56.5 ± 10.0 cm^2, $p < 0.05$) were significantly lower in the DM group than in the non-DM group.

Exercise tolerance and muscle strength

Exercise tolerance and muscle strength at baseline and after CR in each group are presented in Table 4. At the beginning of CR, the levels of peak VO_2 (13.7 ± 4.0 ml kg^{-1} min^{-1} vs. 16.0 ± 4.7 ml kg^{-1} min^{-1}, $p < 0.05$) and thigh muscle strength (136.3 ± 42.7 Nm kg^{-1} × 100 vs. 162.7 ± 47.9 Nm kg^{-1} × 100, $p < 0.05$) were significantly lower in the DM group than in the non-DM group. No significant differences in HGP (28 ± 9 kg vs. 31 ± 9 kg, NS) were observed between the two groups. At the end of CR, both groups showed significant improvements in exercise tolerance and muscle strength. Improvements in exercise tolerance and muscle strength were identical in the DM and non-DM groups. However, the levels of peak VO_2 (19.4 ± 3.8 ml kg^{-1} min^{-1} vs. 22.9 ± 5.4 ml kg^{-1} min^{-1}, $p < 0.05$) and AT (11.3 ± 2.2 ml kg^{-1} min^{-1} vs. 13.3 ± 3.4 ml kg^{-1} min^{-1}, $p < 0.05$) were still significantly lower in the DM group than in the non-DM group. The levels of thigh Ext muscle strength (164.1 ± 43.3 Nm kg^{-1} × 100 vs. 193.3 ± 51.9 Nm kg^{-1} × 100, $p < 0.05$) and HGP (30 ± 7 kg vs. 35 ± 8 kg, $p < 0.05$) were also significantly lower in the DM group than in the non-DM group.

Correlations between muscle mass, muscle strength, and HbA1c

At the end of CR, the values for thigh muscle strength were correlated with thigh circumference ($r = 0.44$, $p < 0.01$) and MAMA ($r = 0.37$, $p < 0.05$) (Fig. 1). The values of HGP were correlated with thigh circumference ($r = 0.52$, $p < 0.01$), and MAMA ($r = 0.48$, $p < 0.05$) (Fig. 1). The same trends were observed at the beginning of CR [9]. Moreover, the percent change in Ex muscle strength was

Table 2
Comparison of glucose, lipid, and other parameters between the DM and non-DM groups.

	DM group ($n = 37$)		Non-DM group ($n = 41$)	
	Baseline	After	Baseline	After
Fasting blood glucose (mg/dl)	143 ± 57	167 ± 68	103 ± 14	112 ± 20
HbA1c (%) (JDS)	7.0 ± 1.3	7.2 ± 1.4	5.1 ± 0.4	5.2 ± 0.5
LDL-C (mg/dl)	116 ± 37	97 ± 22	124 ± 40	89 ± 16
HDL-C (mg/dl)	48 ± 15	50 ± 14	51 ± 12	49 ± 12
TG (mg/dl)	161 ± 97	168 ± 191	149 ± 66	158 ± 97
Creatinine (mg/dl)	1.4 ± 2.3	1.1 ± 1.3	0.8 ± 0.2	0.8 ± 0.2
CRP (mg/dl)	0.6 ± 1.4	0.6 ± 1.3	0.2 ± 0.2	0.7 ± 2.0

Data are presented as the mean value ± SD. DM, diabetes mellitus; HbA1c, hemoglobin A1c; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; CRP, C-reactive protein.
* $p < 0.05$ compared with at baseline.
** $p < 0.05$ compared with the DM group at baseline.
# $p < 0.05$ compared with the DM group after 6 months.

Table 3
Comparison of anthropometric parameters between the DM and non-DM groups.

	DM group ($n = 37$)		Non-DM group ($n = 41$)	
	Baseline	After	Baseline	After
Body mass index (kg m^{-2})	23.3 ± 2.7	22.6 ± 1.9	23.4 ± 2.9	23.7 ± 2.5
Lean body weight (kg)	48.4 ± 9.8	45.2 ± 5.2	49.4 ± 7.7	49.6 ± 7.3
Waist circumference (cm)	83.4 ± 8.3	80.2 ± 5.7*	84.5 ± 7.8	82.2 ± 6.7*
Thigh circumference (cm)	47.2 ± 4.3	47.3 ± 2.5	48.9 ± 4.1	50.7 ± 3.7*,#
Arm forced circumference (cm)	28.3 ± 2.7	28.4 ± 1.6	29.0 ± 2.6	30.0 ± 2.4*,#
Mid-upper arm muscle circumference (cm)	24.9 ± 2.4	25.0 ± 1.8	25.7 ± 2.5	26.5 ± 2.4*,#
Mid-upper arm muscle area (cm^2)	49.7 ± 9.5	49.9 ± 7.1	53.2 ± 10.3	56.5 ± 10.0*,#

Data are presented as the mean value ± SD. DM, diabetes mellitus.
* $p < 0.05$ compared with at baseline.
# $p < 0.05$ compared with the DM group after 6 months.

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Table 4
Comparison of exercise tolerance and muscle strength between the DM and non-DM groups.

	DM group ($n=37$)		Non-DM group ($n=41$)	
	Baseline	After	Baseline	After
Anaerobic threshold (ml kg^{-1} min^{-1})	8.3 ± 1.6	11.3 ± 2.2*	9.7 ± 2.7	13.3 ± 3.4$^{*,\#}$
Peak VO_2 (ml kg^{-1} min^{-1})	13.7 ± 4.0	19.4 ± 3.8*	16.0 ± 4.7**	22.9 ± 5.4$^{*,\#}$
Anaerobic threshold workload (W)	34 ± 15	52 ± 21*	39 ± 20	66 ± 22$^{*,\#}$
Peak workload (W)	73 ± 23	107 ± 21*	81 ± 29	124 ± 29$^{*,\#}$
Knee extension (Nm kg^{-1} × 100)	136.3 ± 42.7	164.1 ± 43.3*	162.7 ± 47.9**	193.3 ± 51.9$^{*,\#}$
Knee flexion (Nm kg^{-1} × 100)	80.0 ± 26.7	102.4 ± 30.3*	91.2 ± 29.2	115.1 ± 30.7*
Power of hand grip (kg)	28 ± 9	30 ± 7*	31 ± 9	35 ± 8$^{*,\#}$

Data are presented as the mean value ± SD. DM, diabetes mellitus.
* $p<0.05$ compared with at baseline.
** $p<0.05$ compared with the DM group at baseline.
$^{\#}$ $p<0.05$ compared with the DM group after 6 months.

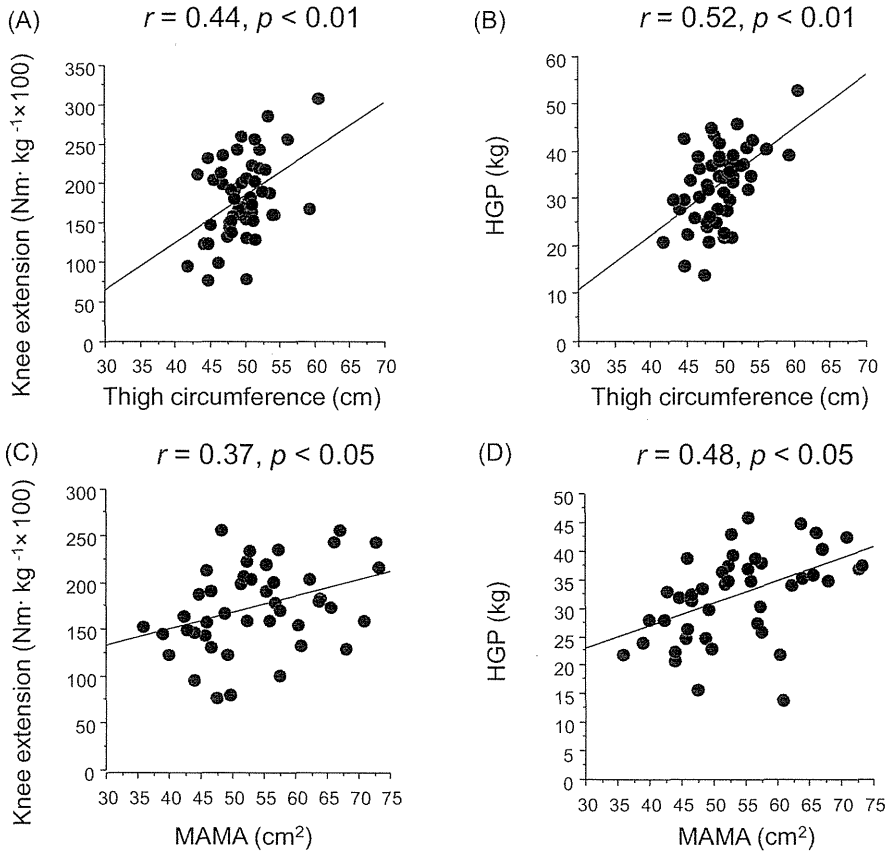

Fig. 1. Correlations between muscle strength and muscle mass. At the end of cardiac rehabilitation, the levels of muscle strength of thigh were correlated with thigh circumference ($r=0.44$, $p<0.01$) (A) and MAMA ($r=0.37$, $p<0.05$) (C). The values of HGP were correlated with thigh circumference ($r=0.52$, $p<0.01$) (B), and MAMA ($r=0.48$, $p<0.05$) (D). MAMA, mid-upper arm muscle area; HGP, hand grip power.

correlated with MAMA ($r=0.47$, $p<0.005$) and HbA1c ($r=-0.41$, $p<0.05$) (Fig. 2).

Discussion

In the present study, we demonstrated that: (1) the levels of muscle strength and exercise tolerance at the beginning and end of CR were significantly lower in the DM group than in the non-DM group; (2) exercise tolerance and muscle strength after CR were significantly improved in both groups; (3) muscle mass was significantly increased after CR in the non-DM group, but not in the DM group; and (4) percent change in muscle strength was correlated with that of HbA1c in patients undergoing CR after CABG. Our group and others previously reported a relationship between muscle strength and peak VO_2 in patients with cardiovascular disease [15,16]. However, to the best of our knowledge, this is the first report to simultaneously demonstrate the effects of CR on muscle mass, muscle strength, and exercise tolerance, and to compare DM and non-DM patients undergoing CR after CABG.

CR is described as a class I recommendation in most contemporary cardiovascular clinical practice guidelines. Following CR, patients show increased exercise tolerance and muscle strength, which have proven to be the strongest predictors of the risk of death among subjects both with and without known cardiovascular disease [17,18]. Boulé et al. reported in a meta-analysis that

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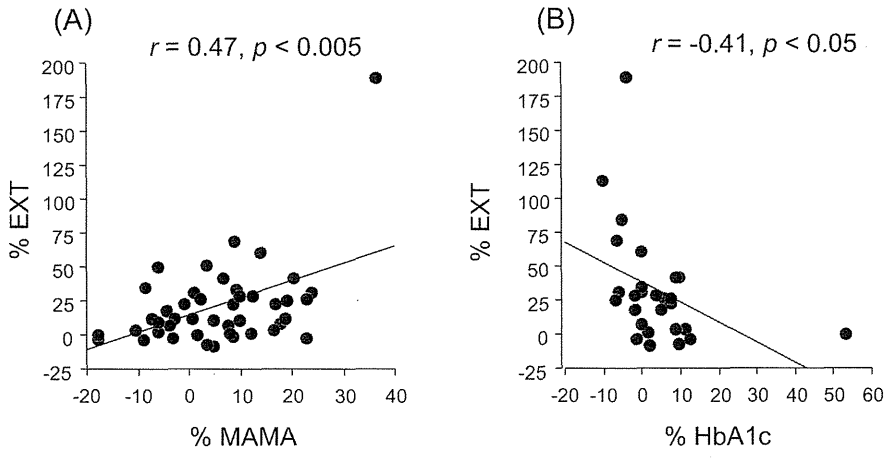

Fig. 2. Correlations between percent change in muscle strength and those in MAMA and HbA1c. A significant relation between the percent change in the muscle strength and those in MAMA was observed ($r = 0.47$, $p < 0.005$) (A). A significant inverse relation between the percent change in the muscle strength and those in HbA_1c was observed ($r = -0.41$, $p < 0.05$) (B). % EXT, percent change of knee extension; % MAMA, percent change of mid-upper arm muscle area; % HbA_1c, percent change of hemoglobin A_1c.

structured exercise training in DM patients achieved an 11.8% increase in peak VO_2 [19]. This is particularly important, because an improvement in peak VO_2 of 1.44 ml kg^{-1} min^{-1} was equivalent to a 7.9% reduction in overall mortality [20]. Moreover, exercise has many potential benefits, including not only improving exercise tolerance, but also improving glucose metabolism, insulin signaling, lipid profile, endothelial function, and blood pressure, reducing vascular inflammation and facilitating weight maintenance [7]. Either aerobic or resistance training alone improves glycemic control in DM patients, however, a combination of both may be more beneficial for improving risk factors than each alone [18]. Williams et al. have reported a combination of aerobic and resistance training exercise improved through neuromuscular adaptation, muscle fiber hypertrophy, and increased muscle oxidative capacity [21]. A previous study demonstrated the beneficial effects of resistance training on muscle mass and strength in chronic heart failure [18]. We also reported that CR with aerobic and resistance training had beneficial effects not only on the modification of metabolic risk factors, but also on improvement in exercise tolerance and muscular strength in patients with metabolic syndrome following CABG [12].

Levels of exercise tolerance and muscle strength were lower in DM than in non-DM patients in the present study. A previous report showed that endothelial dysfunction associated with high glucose levels is caused by the increased production of vasoconstrictor prostanoids as a consequence of protein kinase C activation [22]. Other studies have demonstrated that DM patients have impaired metabolism of both glucose and fatty acids in skeletal muscles. In addition, the bioenergetic capacity of skeletal muscle mitochondria was found to be impaired in DM patients [23]. We previously observed a significant inverse relationship between fasting glucose levels and thigh muscle strength at the beginning of CR in DM patients after CABG [9].

The DM group showed no increase in muscle mass such as MAMA and thigh circumference (Table 3), both of which correlated with thigh muscle strength and HGP (Fig. 1). Vergès et al. reported that the effects of CR on exercise capacity were significantly lower in DM than in non-DM patients, and the response to CR was influenced by blood glucose levels [7]. Moreover, we showed a significant inverse relationship between percent change for HbA1c and that for thigh muscle strength in the DM group (Fig. 2). Park et al. demonstrated that functional muscle quality was relatively low in DM patients. Furthermore, long duration of diabetes and poor glycemic control were associated with deterioration in muscle quality. Diabetes with poor glycemic control is related to the presence and severity of peripheral neuropathy and inflammatory cytokines, which have detrimental effects on muscle function [8]. Chronic hyperglycemia induces a condition of oxidative stress that is causally involved in the development of skeletal muscle depletion [24]. The increased production of reactive oxygen species induced by hyperglycemia has also been suggested to be involved in the redox regulation of glucose transport in skeletal muscle [25]. Hyperglycemia leads to the production of Amadori products between glucose and reactive amino groups of serum proteins [26]. These products undergo further irreversible reactions to form advanced glycation end products that promote insulin resistance and trigger inflammation, which leads to diabetic vascular complications [26]. The DM group had 11.0 ± 6.7 years' duration of DM history in the present study, and the prevalence of microvascular complications, including retinopathy, nephropathy, and neuropathy was 38%. These data may explain the mechanisms by which improvements in muscle mass and strength, and exercise tolerance, were impaired in the DM group. Thus, not only exercise but also glycemic control may be important in improving muscle structure.

Several studies have shown a U-shaped association between BMI and mortality. Increased risk was independent of abdominal and general obesity, and lifestyle and cardiovascular risk factors such as blood pressure and lipid levels were related to early cardiovascular morbidity and mortality. Additionally, Heitmann et al. reported that this risk was related more to thigh than waist circumference [13]. A study in a cohort of community-dwelling Japanese elderly demonstrated that low arm muscle area was an independent risk factor for 2-year mortality [27]. We would like to clarify whether arm muscle area after CR can predict morbidity and mortality in DM patients after CABG.

There are some limitations to the present study. First, because this was a single-center study with a small sample size, studies of larger sample size are required to confirm these findings. Second, the exercise session at the outpatient clinic was encouraged once a week with at least 2 exercise sessions at home. However, while the mean number of CR sessions in hospital was 16–18 times, we have no data regarding home-based exercise frequency and intensity for either group, and we need to assess the effects of exercise frequency and intensity in a future study. The program used in this study may not have been sufficiently rigorous to alter parameters such as glucose control and lipid profiles. Third, we enrolled patients undergoing CR after CABG. Therefore, the results may not necessarily be representative of all DM patients with CAD. In a future study,

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we need to investigate DM patients undergoing percutaneous intervention and/or those with acute coronary syndrome. Finally, we need to investigate whether different treatments, including intensive glucose control and a combination of aerobic and resistance training, can enhance muscle mass and ameliorate future cardiovascular events and long-term mortality in DM patients after CABG.

Conclusions

Patients with DM had lower muscle strength and lower exercise tolerance than non-DM patients at the beginning of CR after CABG. Both groups showed improved exercise tolerance and muscle strength after undergoing CR. However, DM patients had lower muscle mass, lower muscle strength, and lower exercise tolerance than non-DM patients at the end of CR. Moreover, improvement in muscle strength may be influenced by changes in muscle mass and high glucose levels in DM patients. Further studies are needed to assess whether a CR program including muscle mass intervention and aggressive glucose control would improve muscle mass and ameliorate future cardiovascular events in DM patients after CABG.

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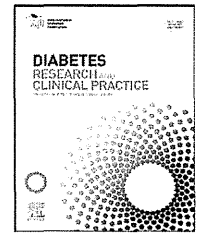

High levels of very long-chain saturated fatty acid in erythrocytes correlates with atherogenic lipoprotein profiles in subjects with metabolic syndrome

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ABSTRACT

Aim: Very long chain saturated fatty acid (VLCFA) levels in erythrocytes are associated with metabolic syndrome (MS). However, the relationship between levels of the VLCFA ligonoceric acid (C24:0) in erythrocytes and the atherogenic lipoprotein profiles and inflammatory state in MS remain unclear.
Methods: Based on the International Diabetes Federation (IDF) definition of MS, 195 apparently healthy males were assigned to either an MS group ($n = 38$) or a non-MS group ($n = 157$). Fatty acid composition of erythrocytes was determined by gas liquid chromatography.
Results: Erythrocytes from the MS group had a significantly higher level of C24:0 than cells from the non-MS group ($4.06 \pm 0.48\%$ versus $3.88 \pm 0.34\%$; $p = 0.03$). C24:0 levels were significantly correlated with several components of MS. The C24:0 levels showed a significant negative correlation with LDL and HDL particle size. Multivariate linear regression analysis showed that C24:0 levels were independently correlated with LDL particle size after adjusting for age and each MS criterion. C24:0 levels were also positively correlated with log-transformed high-sensitivity CRP levels ($p = 0.04$).
Conclusion: C24:0 levels in erythrocytes are associated with specific atherogenic lipoprotein profiles and inflammation status in subjects with MS.

1. Introduction

Metabolic syndrome (MS) is a constellation of metabolic risk factors that includes increased waist circumference, atherogenic dyslipidemia, elevated blood pressure, and elevated blood glucose associated with insulin resistance [1,2]. Several meta-analyses have shown that MS is associated with an approximately 2-fold increased risk of cardiovascular disease [3–5]. One of the characteristic phenotypes of MS is the accumulation of fat in adipose tissue and release of free fatty acids (FFAs) into the circulation. An excessive influx of FFAs

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into muscles and the liver leads to insulin resistance. Several studies have suggested a relationship between plasma fatty acid composition and each of the MS components, including insulin resistance, glucose intolerance, hypertension, and serum lipoprotein disorders [6,7].

Although saturated very long chain fatty acids (VLCFAs) are minor fatty acid components in human tissues and the bloodstream, associations between C26:0 levels, a VLCFA, in erythrocytes and risks of cardiovascular diseases have been observed [8]. We have also reported that absolute C26:0 levels in whole blood were significantly associated with several features of MS [9]. However, levels of C26:0 in the circulation are so low that it is relatively complicated to measure C26:0 levels in clinical settings. Therefore, we confirmed the association between MS and the levels of another saturated VLCFA, lignoceric acid (C24:0), which were measured by a simple established method [10].

Few studies have investigated possible correlations between saturated VLCFA levels and precise atherogenic lipoprotein profiles and systemic inflammatory states, both of which are important MS atherogenic features. Here, we found that a high level of C24:0, but not other fatty acids, in erythrocytes was significantly correlated with small LDL and HDL particles, which are specific components of atherogenic lipoprotein profiles, and high levels of high-sensitivity C-reactive protein (hs-CRP), which indicates systemic inflammation. These results suggest that measuring the level of C24:0 VLCFA in erythrocytes may be a useful marker to evaluate MS atherogenicity.

2. Materials and methods

2.1. Study subjects

We studied 195 consecutive and apparently healthy male subjects who underwent a medical check-up at the Nagasu-Kashiwado Clinic from December 2004 to January 2005. All subjects gave informed consent and the study was approved by the local ethical committee. We excluded patients who were receiving any medicines for diabetes mellitus or dyslipidemias and subjects with high levels of hs-CRP (more than 1 mg/l). Blood pressure (BP) was measured with a standard mercury sphygmomanometer after the subjects had rested for more than 5 min. The mean of two measurements of systolic and diastolic BP while sitting was used. Height and weight were measured using an automated scale, and body mass index was calculated as the weight in kilograms divided by the square of height in meters. Waist circumference was determined by measurements around the umbilical area while standing straight and after expiration.

Study subjects were divided into an MS group and a non-MS group according to the International Diabetes Federation (IDF) definition of MS [2]. Briefly, subjects with MS were defined as having a waist circumference of ≥85 cm plus two or more of the following factors: (1) elevated concentration of triglycerides (TG > 150 mg/dl) or specific treatment for this lipid abnormality; (2) reduced concentration of high density lipoprotein cholesterol (HDL-C < 40 mg/dl) or specific treatment for this lipid abnormality; (3) elevated BP: systolic BP > 130 mmHg or diastolic BP > 85 mmHg or treatment for previously diagnosed hypertension; and (4) elevated fasting plasma glucose (FPG concentration > 100 mg/dl) or previously diagnosed type 2 diabetes.

2.2. Blood sampling

Whole blood samples were drawn after overnight fasting. Serum levels of total cholesterol (TC), TG, and HDL-C were measured by standard enzymatic methods (Kainos, Tokyo, Japan) and low-density lipoprotein cholesterol (LDL-C) values were calculated using the Friedewald formula [11]. Plasma glucose concentrations were determined by the glucose oxidase method (Kainos, Tokyo, Japan) and serum insulin levels were measured according to a double antibody technique (Dainabot, Tokyo, Japan). HbA1c (%) was measured with previously standardized Japanese HbA1c and measurement methods (NGSP). The homeostasis model assessment for insulin resistance (HOMA-IR) was calculated using the following formula: fasting glucose (mmol/l) × fasting insulin (mU/l)/22.5, which was rearranged from the formula originally proposed by Matthews et al. [12].

2.3. Measurement of fatty acid composition in erythrocytes

Total lipids were extracted from erythrocytes by the method of Folch, and fatty acids were directly transmethylated with 14% boron trifluoride methanol solution (Sigma–Aldrich Japan, Tokyo, Japan) at 90 °C for 90 min. Fatty acids were measured using a GC-FID system (6890 N; Agilent Technologies, Tokyo, Japan) equipped with a fused silica capillary column (Omegamax 250; 30 m × 0.25 mm i.d.; 0.25 μm film thickness; Supelco, USA) using tricosanoic acid (C23:0) methyl ester as an internal standard. The injector and detector temperatures were both set at 270 °C and the column temperature was held at 205 °C. Helium was used as the carrier gas at a flow rate of 2.0 ml/min with a split ratio of 50:1 [10].

2.4. Measurement of lipoprotein profiles

Average LDL and HDL particle diameters (nm) were obtained from LDL and HDL peak times with a dual detector, high performance liquid chromatography (HPLC) system with two tandem-connected TSKgel LipopropakXL columns (300 mm × 7.8 mm; Tosoh, Japan) from Skylight Biotech, Inc. (Akita, Japan), as previously described [13,14].

2.5. Statistical analysis

Continuous variables were expressed as mean ± SD and categorical variables were reported as percentages. Statistical differences between the groups were analyzed by Welch's test and chi-square tests. Levene's test was used to assess the equality of variances in different samples. Correlations between two variables were determined by simple linear regression analysis. Multiple linear regression analysis was used to determine the associations between erythrocyte C24:0

levels and LDL particle sizes, HDL particle size or hs-CRP levels independently related to the MS components. Statistical analysis used StatView software (Version 5.0 for Windows, SAS Institute, Cary, NC). p-Values < 0.05 were considered statistically significant.

3. Results

3.1. Characteristics of the study subjects

The characteristics of the subjects in the present study are shown in Table 1. The two groups were not significantly different in terms of age. Compared with the non-MS group, the MS group had significantly higher body mass index (BMI) ($p < 0.001$), waist circumference ($p < 0.001$), systolic BP ($p < 0.001$), diastolic BP ($p < 0.001$), and mean BP ($p < 0.001$). In the MS group, plasma TG levels were significantly increased ($p = 0.005$) and HDL-C levels were significantly decreased ($p < 0.001$) compared with the non-MS group. Plasma TC and LDL-C levels were not significantly different between the two groups. In the MS group, FPG ($p < 0.001$), insulin ($p < 0.001$), HbA1c ($p = 0.01$), HOMA-IR ($p < 0.001$), and hs-CRP ($p = 0.03$) were significantly increased compared with the non-MS group.

3.2. Comparison of erythrocyte fatty acid composition in the MS and non-MS groups

Table 2 shows the fatty acid composition of erythrocytes. In the MS group, erythrocyte levels of C18:0 (stearic acid), and C24:0 (lignoceric acid) were significantly higher than in the non-MS group ($17.6 \pm 1.4\%$ versus $17.2 \pm 1.0\%$, $p = 0.04$; and $4.06 \pm 0.48\%$ versus $3.88 \pm 0.34\%$, $p = 0.03$, respectively). Conversely, MS group erythrocytes had significantly lower levels of C18:1n-7 (vaccenic acid) than erythrocytes from the non-MS group ($1.30 \pm 0.16\%$ versus $1.38 \pm 0.15\%$, $p = 0.005$).

3.3. Correlations between C24:0 and MS risk factors

The correlations between erythrocyte C24:0 levels and the components of MS are shown in Table 3. C24:0 levels were positively correlated with BMI ($r = 0.227$, $p < 0.001$) and systolic BP ($r = 0.158$, $p = 0.03$), plasma LDL-C ($r = 0.167$, $p = 0.02$), and TG ($r = 0.176$, $p = 0.01$). C24:0 levels were negatively correlated with, HDL-C ($r = -0.186$, $p = 0.009$). There was no significant correlation between C24:0 levels and age, plasma TC, fasting plasma glucose, fasting insulin levels, or HOMA-IR.

3.4. Correlation of C24:0 with LDL and HDL particle size

We explored the correlation between erythrocyte C24:0 levels and LDL and HDL particle size (Fig. 1). C24:0 levels were inversely correlated with both LDL and HDL particle diameter. The levels of other fatty acids however, were not significantly correlated with LDL and HDL particle size. After adjusting for each MS criterion (waist circumference, systolic BP, FPG, TG, and HDL-C) and age, C24:0 levels were still independent variables associated with LDL particle size ($p = 0.04$), but not HDL particle size ($p = 0.12$).

3.5. Correlation between C24:0 and hs-CRP

Fig. 2 shows that the level of C24:0 in erythrocytes was significantly correlated with log-transformed hs-CRP levels ($r = 0.15$, $p = 0.04$). After adjusting for each MS criterion (waist circumference, systolic BP, FPG, TG, and HDL-C) and age, there was no significant association between log-transformed C24:0 levels and hs-CRP levels ($p = 0.36$).

4. Discussion

The level of C24:0 was significantly higher in erythrocytes from the MS group than the non-MS group, and C24:0 levels

Table 1 – Characteristics of study subjects.

	Non-MS	MS	F statics	p value
	$n = 157$	$n = 38$		
Age (years)	50.2 ± 9.9	50.1 ± 8.6	0.11	NS
Body mass index (kg/m^2)	23.2 ± 2.5	27.2 ± 3.3	0.29	<0.001
Waist circumference (cm)	83.4 ± 6.5	95.0 ± 6.3	0.32	<0.001
Systolic blood pressure (mmHg)	127 ± 15	139 ± 15	0.58	<0.001
Diastolic blood pressure (mmHg)	80 ± 10	87 ± 11	0.53	<0.001
Current smoker (%)	69 (44)	16 (43)		NS
Total cholesterol (mg/dl)	194 ± 35	190 ± 39	0.05	NS
Triglycerides (mg/dl)	111 ± 44	130 ± 32	0.33	0.005
HDL-cholesterol (mg/dl)	58 ± 12	47 ± 8	0.009	<0.001
LDL-cholesterol (mg/dl)	77 ± 18	82 ± 15	0.15	NS
Blood glucose (mg/dl)	99 ± 13	108 ± 14	0.46	<0.001
Insulin (mU/l)	4.6 ± 2.7	9.4 ± 6.7	<0.001	<0.001
HOMA-IR	1.2 ± 0.9	2.6 ± 2.1	<0.001	<0.001
HbA1c (NGSP)(%)	5.7 ± 0.4	6.0 ± 0.8	0.004	0.01
Hs-CRP (mg/l)	0.86 ± 1.41	1.66 ± 2.09	0.002	0.03

Values are mean ± SD. MS, metabolic syndrome; HDL, high density lipoprotein; LDL, low density lipoprotein; HOMA-IR, homeostasis model assessment for insulin resistance; Hs-CRP, high sensitive C reactive protein.

Table 2 – Proportion of fatty acid (%) in erythrocytes from MS and non-MS subjects.

	Non-MS $n = 157$	MS $n = 38$	F statics	p value
C14:0 (myristic acid)	0.21 ± 0.05	0.22 ± 0.06	0.04	NS
C16:0 (palmitic acid)	20.4 ± 1.3	20.9 ± 1.6	0.12	0.08
C16:1*n*-7 (palmitoleic acid)	0.44 ± 0.07	0.46 ± 0.08	0.70	NS
C18:0 (stearic acid)	17.2 ± 1.0	17.6 ± 1.4	0.004	0.04
C18:1*n*-9 (oleic acid)	12.4 ± 0.82	12.3 ± 0.79	0.80	NS
C18:1*n*-7 (vaccenic acid)	1.38 ± 0.15	1.30 ± 0.16	0.87	0.005
C18:2*n*-6 (linoleic acid)	8.30 ± 0.97	8.05 ± 1.06	0.57	NS
C18:3*n*-3 (α-linolenic acid)	0.13 ± 0.03	0.12 ± 0.03	0.86	NS
C20:0 (arachic acid)	0.35 ± 0.04	0.34 ± 0.04	0.78	NS
C20:1	0.42 ± 0.09	0.43 ± 0.10	0.28	NS
C20:3*n*-6 (dihomo-γlinolenic acid)	1.16 ± 0.18	1.22 ± 0.17	0.80	NS
C20:4*n*-6 (arachidonic acid)	10.9 ± 1.6	10.5 ± 1.8	0.44	NS
C20:5*n*-3 (eicosapentaenotic acid)	1.66 ± 0.77	1.57 ± 0.74	0.78	NS
C22:0 (behenic acid)	1.22 ± 0.15	1.25 ± 0.16	0.58	NS
C22:1	0.13 ± 0.15	0.11 ± 0.12	0.63	NS
C22:4*n*-6	1.56 ± 0.41	1.53 ± 0.51	0.03	NS
C22:5*n*-6 (*n*-6 docosapentaenoic acid)	0.28 ± 0.11	0.26 ± 0.07	0.71	NS
C22:5*n*-3 (*n*-3 docosapentaenoic acid)	2.27 ± 0.38	2.16 ± 0.42	0.69	NS
C22:6*n*-3 (docosahexaenoic acid)	7.01 ± 1.43	6.84 ± 1.78	0.23	NS
C24:0 (lignoceric acid)	3.88 ± 0.34	4.06 ± 0.48	0.02	0.03
C24:1*n*-9 (nervonic acid)	4.17 ± 0.39	4.19 ± 0.44	0.37	NS

Values are mean ± SD.

were significantly associated with several components of MS. Additionally, we found that the increased level of C24:0, but not other fatty acids, in erythrocytes was significantly correlated with small LDL and HDL particle size, which are specific components of atherogenic lipoprotein profiles. Increased C24:0 levels were also positively correlated with systemic inflammation as indicated by hs-CRP levels.

Fatty acid beta-oxidation occurs in both mitochondria and peroxisomes. Long chain fatty acids (C16–C20) are primarily oxidized in mitochondria, whereas peroxisomes are involved in the beta-oxidation of VLCFAs (>C20) [15]. Peroxisomal dysfunction gives rise to an over-accumulation of VLCFAs in the body as a whole [16]. VLCFAs accumulate in the plasma, membranes of erythrocytes, and/or tissues of patients with inherited peroxisomal diseases, which are characterized by progressive demyelination and adrenal insufficiency [17–19]. X-adrenoleukodystrophy (X-ALD), the most common peroxisomal disorder, is associated with increased levels of saturated VLCFAs (>C22:0) [16]. Treatment with the potent and selective histone deacetylase inhibitor normalized the levels of VLCFAs in skin fibroblasts from X-ALD patients by increasing the peroxisomal C24:0 beta-oxidation activity [20]. Peroxisomal dysfunction plays an important role in aging-related diseases [21], and, furthermore, a recent report suggested that peroxisome-related alterations and increased VLCFAs may contribute to the progression of Alzheimer's disease [22].

Table 3 – Correlations of the proportion of C24:0 with risk factors of metabolic syndrome.

	r	t-Score	p-Value
Age	0.084	1.17	NS
Body mass index	0.227	3.24	0.001
Waist circumference	0.258	3.71	<0.001
Systolic blood pressure	0.158	2.23	0.03
Total cholesterol	0.139	1.95	NS
HDL-cholesterol	−0.186	−2.62	0.009
LDL-cholesterol	0.167	2.31	0.02
Triglycerides	0.176	2.49	0.01
Fasting plasma glucose	0.066	0.92	NS
Fasting serum insulin	0.125	1.75	NS
HOMA-IR	0.099	1.38	NS

HDL, high density lipoprotein, LDL, low density lipoprotein, HOMA-IR, homeostasis model assessment for insulin resistance.

The expression of enzymes involved in fatty acid synthesis and elongation may contribute to the accumulation of VLCFAs. In particular, ELOVL1 and ELOVL3 have chain length specificity toward VLCFA [23,24], and silencing of ELOVL1 reduces elongation of C22:0–C26:0 and lowers C26:0 levels in X-ALD fibroblasts [23]. ELOVL3 mediates the elongation of C22:0–C24:0 and C24:0–C26:0 in vivo [24]. It has been reported that ELOVL3 expression regulates diet-induced obesity, hepatic lipogenic gene expression, and hepatic TG content [25]. Several studies, including our own, have demonstrated the association of saturated VLCFA levels with the risks of cardiometabolic syndrome [8,9]. In this study, the accumulation of C24:0 was also associated with MS. Taken together, MS may be associated with an imbalance between the synthesis and metabolism of saturated VLCFAs.

LDL and HDL particles associated with MS tend to be small and dense [26]. Smaller LDL particles are more atherogenic than larger LDL as they may filter more readily into the arterial wall and are more prone to atherogenic modifications [27]. Small, dense HDL sub-fractions are increased in MS and are associated with elevated oxidative stress and insulin resistance [28]. However, the association between various fatty acid

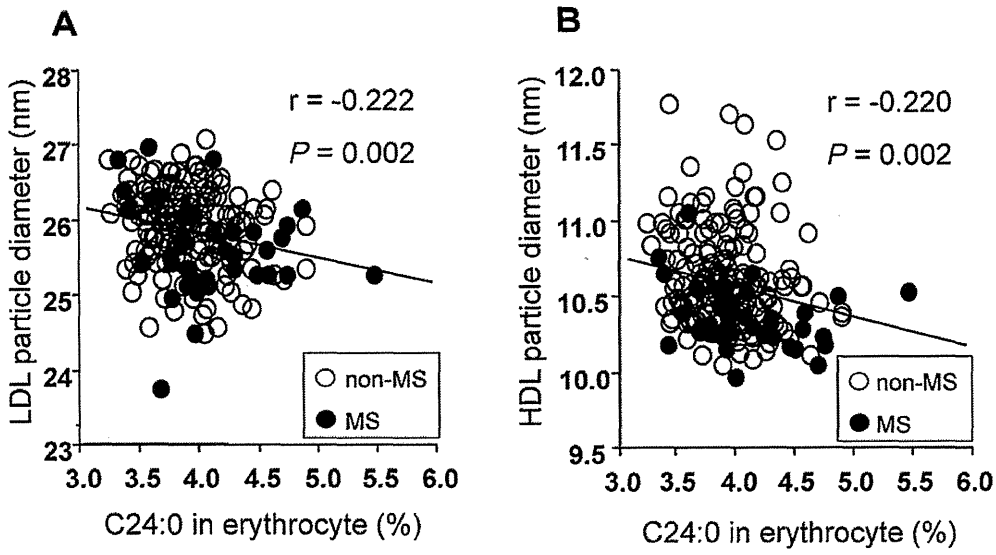

Fig. 1 – Correlation of the level of C24:0 in erythrocyte with low density lipoprotein (LDL) particle diameter (A) and high density lipoprotein diameter (B).

components and precise atherogenic lipoprotein profiles remains unclear.

Several studies have reported associations between dietary fatty acids and atherogenic lipoproteins. Consumption of dietary *trans* fatty acids is associated with an increase in small, dense LDL particles [29]. In animal experiments, HDL particle size was significantly smaller in male Hartley guinea pigs that were fed *trans* fatty acids compared with guinea fed other diets [30]. Dietary unsaturated fats similarly reduce LDL size relative to saturated fats, although the composition of dietary fat is not a major factor affecting LDL size [31]. However, in this study population, only a high level of saturated VLCFA C24:0, but not other fatty acids, in erythrocytes showed a significant correlation with both small LDL and HDL particle size. This indicates that the accumulation of VLCFAs may play a crucial role in the pathogenesis of atherosclerosis.

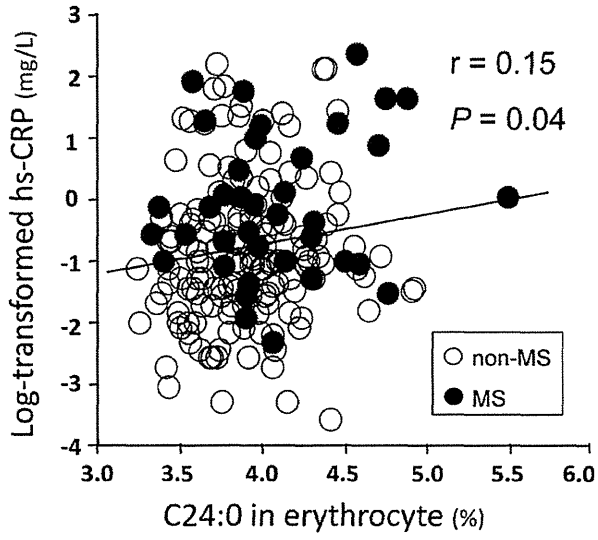

Fig. 2 – Correlation of the level of C24:0 in erythrocyte with log-transformed high sensitive C reactive protein (hs-CRP).

We also found a significant association between increased erythrocyte C24:0 level and high hs-CRP levels, indicating that the accumulation of C24:0 may interact with the inflammatory state in MS. Long chain saturated fatty acids (>C12:0) have relatively high melting points; therefore, increased levels of saturated fatty acids have the potential to reduce cell membrane fluidity. Reduced erythrocyte membrane fluidity may be associated with endothelial dysfunction and increased oxidative stress [32,33]. We recently reported that macrophages with accumulated saturated VLCFAs obtained from mice with peroxisome dysfunction produced several inflammatory cytokines and increased oxidative stress [34]. A recent report showed the alteration of long-chain fatty acid composition in plasma and erythrocytes due to higher levels of chronic oxidative stress are associated with the pathophysiology of depression [35]. These results suggest that the accumulation of saturated VLCFAs in various cells and organs may be involved in inflammation and oxidative stress during the pathogenesis of MS.

This study has several limitations. First, we have no data available on the dietary fatty acid intake of our subjects. The effects of fatty acid intake on the accumulation of saturated VLCFA in erythrocytes will require additional study. Second, we did not assess plasma parameters associated with peroxisomal beta-oxidation or the enzyme activity related to the elongation of fatty acids. Therefore, additional studies are needed to clarify the contribution of erythrocyte VLCFAs to MS.

5. Conclusion

We have confirmed the association between a saturated VLCFA (C24:0) and MS. In addition, we found that a high level

of C24:0, but not other fatty acids, in erythrocytes was significantly correlated with atherogenic lipoprotein profiles and an inflammation marker. In conclusion, measuring the level of C24:0 in erythrocytes may be a useful marker to evaluate MS atherogenicity.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

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原　著

生活習慣病患者における歩数計を利用したセルフモニタリングによる運動指導は身体活動量を増加させ血管内皮機能の改善につながる

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要　約　高い身体活動量は血管内皮機能を良好な状態に維持し、心血管疾患の罹患率や死亡率を低下させる。歩数計の利用は身体活動量の増加に有効であるが、歩数計を利用したセルフモニタリングによる運動指導の有効性を血管内皮機能の観点から検討したものは極めて少ない。そこで本研究は、生活習慣病患者における歩数計を利用したセルフモニタリングによる運動指導が、身体活動量を変化させ血管内皮機能に影響するか否かについて検討した。【方法】複数の冠危険因子を有する生活習慣病患者27名を対象とした。対象患者は、運動指導前に加速度計付きの歩数計を1-2週間装着し、運動指導前の歩数と運動時間を測定した。運動指導として、歩数計を用いて10,000歩/日、および中等度の運動強度である速歩を多く取り入れて歩行するように指導した。運動指導は観察開始時と開始から2週間後の2回行い、観察期間は4週間とした。血管内皮機能として反応性充血による血管内皮機能指数(RH-PAT index)を測定した。観察期間の前後で臨床的背景因子、RH-PAT index、歩数、運動量、中強度の運動時間を測定した。さらに、各測定項目において観察期間の前後の測定値からその変化量(Δ)を算出し、運動効果を評価した。【結果】観察期間の前後でRH-PAT index、歩数、運動量、中強度の運動時間は有意に増加した（それぞれ$P = 0.028$、$P < 0.0001$、$P < 0.0001$、$P < 0.0001$）。さらに各測定項目の変化量について検討すると、ΔRH-PAT indexは、Δ歩数（$r = 0.531$、$P = 0.004$）、Δ運動量（$r = 0.555$、$P = 0.003$）とそれぞれ有意な正の相関関係を示した。【結論】生活習慣病患者における歩数計を利用したセルフモニタリングによる運動指導は、身体活動量を増加させ、血管内皮機能の改善につながると思われた。

キーワード：生活習慣病，歩数計，身体活動量，血管内皮機能
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I. 緒　　言

近年、我が国において生活習慣病を予防するための身体活動量・運動量および体力の基準値が示され、日常的に3METs以上の身体活動を確保し、週23エクササイズ以上の活発な身体活動を行うことが推奨されている[1,2]。この週23エクササイズの身体活動は、歩数に換算すると1日8,000～10,000歩に相当し、平成21年国民健康・栄養調査[3]によると、国民の平均歩数は男性7,011歩、女性5,945歩であることから、推奨されている歩数には達していないのが現状である。さらに、疾病管理されている慢性疾患を有する中高年者については、中等度（3-6METs）の身体活動を1日30分以上、週5回以上行うこと[4]や、動脈硬化性疾患予防ガイドラインでは最大酸素摂取量の約50%

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の有酸素運動を1日30分以上、週3回以上行うこと[5]が推奨されている。したがって、歩数とともに中等度の身体活動量を増加させ、高い身体活動量を確保することが健康増進や心血管疾患の発症予防に重要である。

高い身体活動量が心血管疾患の罹患率や死亡率を低下させることは数々の疫学研究から明らかとなっている[6,7]。高い身体活動量と心血管疾患の罹患率・死亡率低下の間には、良好な体重、インスリン抵抗性、血糖・脂質コントロール、血圧、血管内皮機能の維持、あるいはそれらの改善による効果が示唆されている[8]。中でも血管内皮機能は、高血圧、糖尿病、脂質異常症などの冠危険因子の存在によって動脈硬化発症の第一段階として障害される[9]。血管内皮細胞は様々な生理活性物質を産生・分泌することが明らかとなっているが、特に一酸化窒素（NO）は血管トーヌスの調整、血小板凝集や血管平滑筋増殖の抑制など、抗動脈硬化作用として重要な役割を果たしている[10]。日常的に高い身体活動量を維持することは血管内皮機能を良好な状態に維持し、加齢による血管内皮機能障害を是正すること[11]、さらに血管内皮機能障害は心血管疾患発症の予測因子であること[12,13]が示されている。したがって、身体活動量増加による血管内皮機能の改善は将来的な心血管疾患の罹患率や死亡率を抑制し得る可能性があり、身体活動量を増加させ、その効果判定指標として血管内皮機能を評価することは、予防医学的観点から非常に重要であると考えられる。

身体活動量を増加させるためには歩数計を利用したセルフモニタリングが有効であり、これにより歩数は約2,500歩増加する[14]。さらに、歩数計を利用した身体活動量増加の効果として、収縮期血圧・拡張期血圧[14]、Body mass index（BMI）の低下[14,15]が報告されている。自転車エルゴメーターなどの機械を用いた有酸素運動により血管内皮機能が改善することは数多く示されている[16-21]が、歩数計を利用したセルフモニタリングによる身体活動量増加の効果を血管内皮機能の側面から検討した報告は極めて少ない。そこで本研究は、歩数計を用いたセルフモニタリングによる身体活動量増加の効果判定指標として血管内皮機能に着目し、生活習慣病患者を対象として、歩数計を利用した身体活動量の増加が血管内皮機能に与える影響について検討した。

II. 方　　法

1. 対象

北里大学東病院循環器内科外来、または心臓二次予防センターに通院中で複数の冠危険因子（高血圧、糖尿病、脂質異常症、喫煙、肥満、冠動脈疾患の既往のうち2つ以上）を保有し、口頭にて本研究の意義と測定方法を十分に説明し、書面にて研究に参加する同意が得られた生活習慣病患者27名を対象とした。冠危険因子は、収縮期血圧≧140mmHgまたは拡張期血圧≧90mmHg、あるいは降圧薬を服用している場合を高血圧、空腹時血糖≧126 mg/dLおよびヘモグロビンA1c（HbA1c）≧6.1%（JDS基準）、あるいは糖尿病治療薬を服用している場合を糖尿病、LDLコレステロール（LDL-C）≧140mg/dL・HDLコレステロール（HDL-C）＜40 mg/dL・中性脂肪≧150 mg/dLのいずれかを有する、あるいは高脂血症治療薬を服用している場合を脂質異常症、現在喫煙している場合を喫煙、BMI≧25 kg/m^{2}の場合を肥満、心筋梗塞・狭心症の既往がある場合を冠動脈疾患の既往と定義した。除外基準として、研究参加の同意が得られなかった者、歩行困難な整形外科的疾患・中枢神経疾患を有する者、認知症を有する者、精神疾患を有する者、重症肝不全・腎不全を有する者は対象から除外した。なお、本研究は北里大学医学部B倫理委員会の承認を受けて実施した。

2. 測定項目

1）臨床的背景因子

臨床的背景因子として、年齢、性別、身長、体重、BMI、腹囲、収縮期血圧、拡張期血圧、冠危険因子、服薬状況を調査した。

2）血液生化学検査

血液は採血前日の夜から12時間以上の絶食後に採取し、脂質代謝指標としてLDL-C、HDL-C、中性脂肪、糖代謝指標として空腹時血糖、HbA1c、炎症指標としてC-reactive protein（CRP）を測定した。

3）血管内皮機能

血管内皮機能の測定にはEndo-PAT2000（Itamar Medical Ltd.、Caesarea、Israel）を用いて、反応性充血による血管内皮機能検査（reactive hyperemia peripheral arterial tonometry：RH-PAT）を実施した。RH-PAT検査は、最低4時間以上の絶食後に実施し、検査前の水分摂取はカフェイン、アルコール以外の飲料水の摂取のみ許可した。各被験者は暗い静かな部屋で仰臥位をとり、タオルケットをかけた状態で15分間の安静をとった後にRH-PAT検査を実施した。なお、観察期間の前後でRH-PAT検査を実施する時間帯を被験者ごとに一致させた。

RH-PAT検査は、上腕駆血後再灌流時における指尖脈波の変動をPATプローブにて検出し、コンピュータ解析で定量化するシステムであり、測定方法は先行研究に準じて行った[22,23]。すなわち、各対象者の利き手側の上腕で血圧を測定し、非利き手側の上腕に駆血用カフを巻きつけた。左右の示指にPATプローブを取り付け、ベースラインとして安静時の指尖脈波を5分間測定した後、駆血用カフで収縮期血圧＋60mmHg、または200mmHgの高い方の圧力で5分間駆血した。5分間の駆血後、駆血用カフを解放して駆血解放後の指尖脈波を5分間測定した。RH-PAT検査にて得られた指尖脈波のデータから、パソコンの自動解析により駆血側のベースライン（A）に対する駆血解放後（B）の指尖脈波の比（B/A）、および非駆血側のベースライン（C）に対する駆血解放後（D）の指尖脈波の比（D/C）をそれぞれ算出した。駆血側の指尖脈波の比（B/A）を非駆血側の指尖脈波の比（D/C）で除すことでRH-PAT indexを算出し、解析値とした（図1）。

4）身体活動量

身体活動量の測定には加速度計付きの歩数計である生活習慣記録機Lifecorder GS（LC；SUZUKEN CO.、LTD.、Nagoya、Japan）を用いて、歩数、運動量、中強度（3-6METs）の運動時間を測定した。LCは内蔵された加速度センサーにより、歩数、エネルギー消費量を評価し、さらに独自のアルゴリズムにて運動強度を0-9の10段階の加速度強度に分類する。運動強度は1-3が3METs未満、4-6が3-6METs、7-9が6METs以上に相当する[24]ことから、LCが記録した運動時間のうち、4-6の運動を中強度の運動時間とした。対象者は、LCを入浴時と睡眠時を除いて終日腰部に装着した。装着期間は、対象者の身体活動量を正確に把握可能とされる1週間以上[25,26]とし、その平均値を解析値として用いた。

3．運動指導

運動指導として、各種ガイドライン[1,2,4,5]を参考にして「1日10,000歩を目標に歩くこと」、および「中等度の運動強度である速歩をなるべく多く取り入れること」を、LCを利用しながら実施するよう指導した。さらに、運動記録用紙を配布し、日々の歩数、歩数と速歩に対する運動の達成度をセルフモニタリングすることを指導した。観察期間は4週間とし、初回の運動指導から2週間経過した後、電話による再運動指導を実施した。再運動指導では、配布した運動記録用紙の情報をもとに2週間の平均歩数、運動の達成程度を聴取し、指導した運動が実施できているか否かを調査した。指導した運動が実施できていない場合、歩数と速歩が少しでも目標に近づくよう再度指導した。なお、研究期間中は今まで通りの食生活、服薬を続けてもらうよう全被験者に説明した。

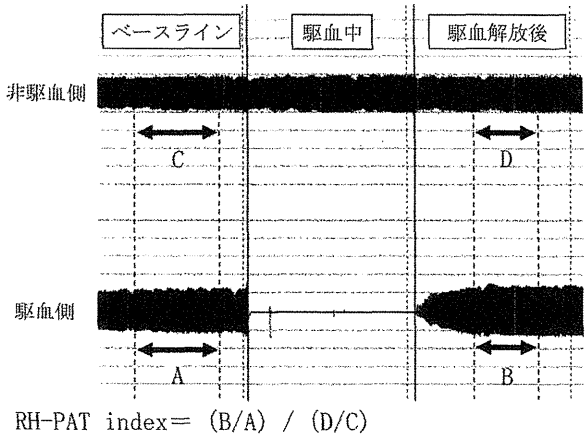

図1　Reactive hyperemia-peripheral arterial tonometry (RH-PAT) indexの算出方法
A：駆血側のベースライン時の指尖脈波、
B：駆血側の駆血解放後の指尖脈波
C：非駆血側のベースライン時の指尖脈波、
D：非駆血側の駆血解放後の指尖脈波
RH-PAT index＝（B/A）/（D/C）