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動脈硬化と心臓リハビリテーション

Atherosclerosis and cardiac rehabilitation — From bedside to bench —

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抄 録

動脈硬化性疾患に対する心臓リハビリテーションは，早期離床，社会復帰，危険因子やQOLの改善のみならず，予後を改善することが明らかにされている．実際に我々も，メタボリックシンドロームを有する冠動脈バイパス術施行患者に対する回復期心臓リハビリテーションは，除脂肪量を減少させることなく腹囲や脂肪重量を有意に減少，運動耐容能や脚筋力を有意に増加，高感度CRPを有意に低下させることを報告した．さらに最近，高齢冠動脈疾患患者に対する維持期心臓リハビリテーションは，冠危険因子の是正やQOLの改善のみならず，長期予後の改善にも有効であることを報告した．これらの動脈硬化性疾患に対する心臓リハビリテーションの有効性の機序を明らかにするために，我々は，動脈硬化モデルマウスを用いて基礎的検討を行った．アポE欠損マウスを，普通食群，高脂肪食群，高脂肪食＋自発運動群に分割し，動脈硬化病変の形成，血管内皮機能，白色および褐色脂肪組織の重量変化，大動脈および血中の炎症レベルを検討した．高脂肪食により，血管内皮依存性弛緩反応は有意に低下し，動脈硬化性病変の形成は有意に増加した．自発運動は，血管内皮依存性弛緩反応を保持し，動脈硬化性病変の形成を有意に抑制した．自発運動により，大動脈組織における炎症性細胞の発現は有意に低下し，血中の炎症性サイトカインやケモカイン濃度も有意に低下した．また，白色脂肪重量が減少し，血中アディポネクチン濃度は有意に増加した．さらに，自発運動と薬物治療（スタチン，アンジオテンシンⅡ受容体拮抗薬）の併用は，自発運動単独または薬物治療単独に比し，動脈硬化性病変の形成を抑制した．以上より，心臓リハビリテーションの中心の一つである運動療法は，組織および血中の炎症を抑制することにより動脈硬化の発症や進展を抑制すること，さらに運動療法と薬物治療を含めた包括的治療が，動脈硬化の発症や進展を抑制に極めて有用であることが基礎的検討からも示唆された．

〔心臓リハビリテーション（JJCR）16（1）：40-43，2011〕

Key words：動脈硬化，自発運動，アポE欠損マウス，炎症

1．はじめに

動脈硬化性疾患に対する心臓リハビリテーションは，早期離床や社会復帰，危険因子やQOLの改善のみならず，予後を改善することが明らかにされている[1]．実際に我々も，メタボリックシンドロームを有する冠動脈バイパス術施行患者に対する回復期心臓リハビリテーションは，除脂肪量を減少させることなく腹囲や脂肪重量を有意に減少，運動耐容能や脚筋力を有意に増加，高感度CRPを有意に低下させることを報告した[2]．さらに最近，高齢冠動脈疾患患者に対する維持期心臓リハビリテーションは，冠危険因子の是正やQOLの改善のみならず，長期予後の改善にも有効であることを報告した[3]．

以上より，運動が動脈硬化性疾患の予防や治療に有用であることは明らかである[4]．これらの動脈硬化性疾患に対する心臓リハビリテーションの有効性の機序を明らかにするために，我々は，動脈硬化モデルマウスを用いて基礎的検討を行った．

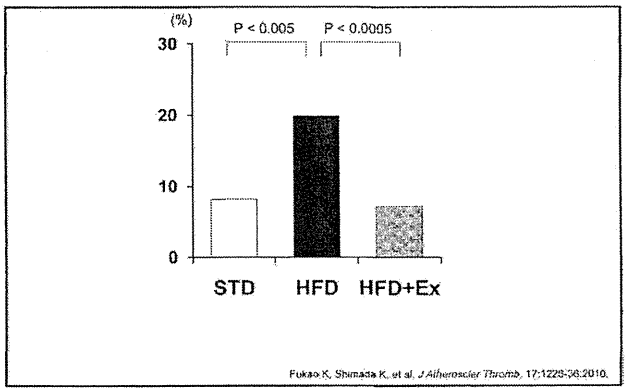

図1　大動脈弁輪部における動脈硬化病変
STD：standard diet, HFD：high fat diet, HFD + Ex：high fat diet + voluntary exercise.

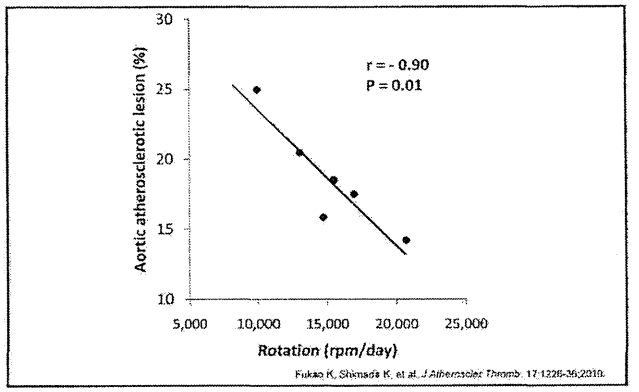

図2　運動量と動脈硬化病変との関連

2. 動脈硬化モデルマウスを用いた基礎的検討

a) 対象と方法

動物を用いた持久性運動は，強制的運動と自発的運動に分類することができる．これまでの動物モデルを用いた研究では，主にトレッドミルや水泳等の強制運動による評価が行われてきた[5, 6]．夜行性であるマウスに対する日中のトレッドミルによる運動，トレッドミル走行スピード低下時の電気刺激，本来泳がないマウスの溺水に対する過度のストレス，さらにその温水環境など，従来の強制運動を用いた動脈硬化に対する評価には，自律神経や温熱効果等の様々な因子が関与している可能性がある．したがって，今回我々は，自発運動を用いて，動脈硬化に対する運動の効果を検討した．

8週齢雄のアポE欠損マウスを，普通食（STD）群，高脂肪食（HFD）群，高脂肪食＋自発運動（HFD + Ex）群に分割した．自発運動は，ホイールケージを使用した．それぞれ10週間後に，体重，白色および褐色脂肪組織の重量変化，血清脂質を測定した．動脈硬化面積は，SUDAN-Ⅳによる大動脈展開標本およびOil red-O染色による大動脈弁輪部標本を作製し計測した．また，アセチルコリンによる血管内皮機能を解析した．さらに，血中のinterleukin（IL）-6，tumor necrosis factor（TNF）-α，macrophage chemoattractant protein（MCP）-1，アディポネクチン測定，大動脈よりRNAを抽出し，realtime-PCR法にて遺伝子発現を確認した[7]．

次に，8週齢雄のアポE欠損マウスを，STD群，HFD群，HFD + Ex群，高脂肪食＋スタチン（HFD + S）群，高脂肪食＋アンジオテンシンⅡ受容体拮抗薬（HFD + ARB）群，高脂肪食＋スタチン＋自発運動（HFD + S + Ex）群，高脂肪食＋ARB＋自発運動（HFD + ARB + Ex）群に分割した．自発運動は，前述と同様にホイールケージを使用し8週間行い，薬物は，浸透圧ポンプを皮下に挿入し8週間持続投与した．動脈硬化性変化は，SUDAN-Ⅳによる大動脈展開標本を用いて評価した．

b) 結　果

HFD + Ex群の10週後の体重は，HFD群に比し有意に低値であったが，体重増加率は3群間で有意差を認めなかった．HFD群およびHFD + Ex群の血清コレステロールとvery low density lipoproteinコレステロールは，STD群に比し有意に高値であったが，HFD群とHFD + Ex群との間には有意差を認めなかった．HFD群の精巣周囲の白色脂肪重量と総白色脂肪重量は，STD群に比し有意に高値であった．HFD + Ex群の精巣周囲の白色脂肪重量と総白色脂肪重量は，HFD群に比し有意に低値であった．HFD群の大動脈弁輪部および大動脈展開標本における動脈硬化面積は，STD群に比し有意に高値であった．HFD + Ex群の大動脈弁輪部および大動脈展開標本における動脈硬化面積は，HFD群に比し有意に低値であった（**図1**）．HFD + Ex群における1日あたりの運動量は，大動脈展開標本における動脈硬化面積と有意な負の相関を認めた（**図2**）．また，HFD + Ex群における血管内皮機能は，HFD群に比し有意に保持されていた．血中のIL-6，TNF-α，MCP-1は，STD群に比しHFD群で有意に高値であり，HFD群に比しHFD + Ex群で低値であった（**図3**）．アディポネクチンは，HFD群に比しHFD + Ex群で有意に高値であった（図3）．大動脈におけるCD68

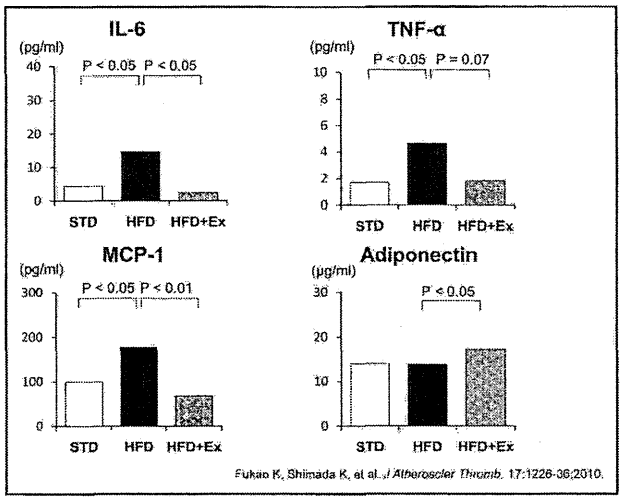

図3　血中サイトカイン・ケモカイン濃度
STD：standard diet, HFD：high fat diet, HFD + Ex：high fat diet + voluntary exercise. IL：interleukin, TNF：tumor necrosis factor, MCP：macrophage chemoattractant protein.

（マクロファージ）CD4（リンパ球）CD11c（樹状細胞）の発現は，STD 群に比し HFD 群で高値であり，HFD 群に比し HFD + Ex 群で低値であった（**図 4**）[7]．

HFD + S 群と HFD + ARB 群の大動脈展開標本の動脈硬化病変形成は，HFD 群に比し有意に減少した．さらに，HFD + S + Ex 群の大動脈硬化病変形成は，HFD + S 群および HFD + Ex 群に比し有意に減少した．同様に，HFD + ARB + Ex 群の大動脈硬化病変形成は，HFD + ARB 群および HFD + Ex 群に比し有意に減少した．

c）考　察

アポ E 欠損マウスに対する高脂肪食負荷は，総コレステロールも含めた血清脂質を有意に増加，血管内皮依存性弛緩反応を有意に低下，動脈硬化性病変の形成を有意に増加させた．同時に，血中の炎症性サイトカインやケモカインは有意に増加し，大動脈組織におけるマクロファージ，リンパ球，樹状細胞の発現は，有意に増加した．一方，同じ高脂肪食負荷を行ったにもかかわらず，自発運動により血管内皮依存性弛緩反応，動脈硬化性病変の形成，血中の炎症性サイトカインやケモカイン，大動脈組織におけるマクロファージ，リンパ球，樹状細胞の発現は低下した．さらに，自発運動は，血中アディポネクチンを増加させた．血清総コレステロールは，自発運動により有意な改善は認められなかったことより，自発運動による血管内皮機能や動脈硬化病変の改善は，血清脂質の改善よりもむしろ大動脈組織および全身の炎症状態を改善することによりもたらされた可能性が考えられた．さらに，大動脈硬化病変は，自発的運動量と有意な負の相関を認めたことは興味深い結果である．

また，スタチンや ARB の投与は，自発運動と同様に大動脈硬化病変の形成を抑制した．スタチンや ARB 投与に自発運動を組合せることは，大動脈硬化病変の形成に対して相加的な効果が期待できる可能性を示した．スタチンや ARB は，心血管イベント発症を有意に減少させることが多くの臨床研究により明らかにされている．両剤は，基礎研究からも抗動脈硬化作用を有することが報告されている．したがって，動脈硬化の遺伝的リスクを有し，さらに食習慣の改善が十分とはいえない状況においても，自発的運動の継続が，抗動脈硬化の観点からも極めて重要であると考えられる．

本研究は，マウスの動脈硬化病変形成を検討してお

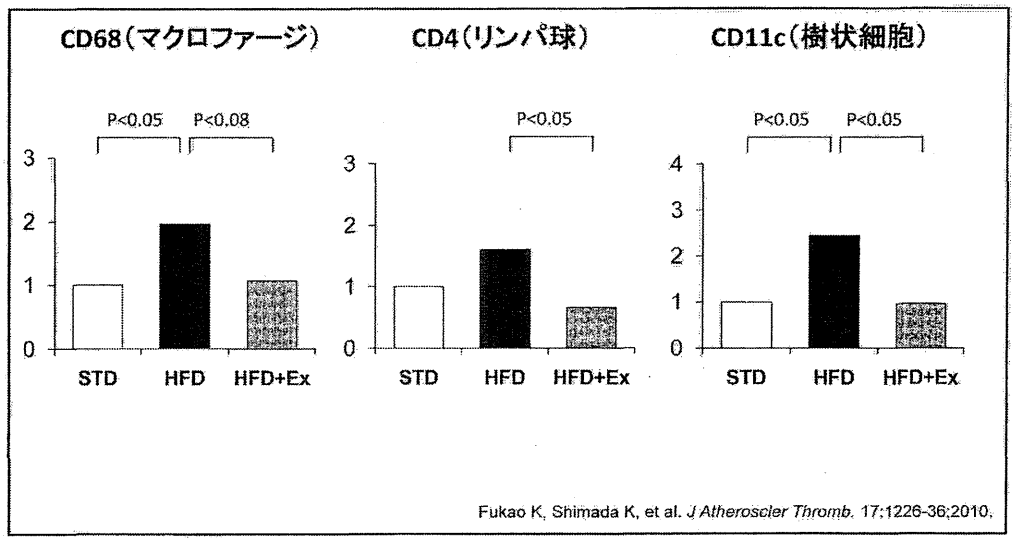

図4　大動脈組織における mRNA 発現
STD：standard diet, HFD：high fat diet, HFD + Ex：high fat diet + voluntary exercise.

り，ヒトを対象とした臨床研究ではない．マウスとヒトでは，血管径が明らかに異なる．また，炎症性細胞の役割も異なる可能性も考えられる．今後，臨床的検討も含めたさらなる研究が必要である．

3. 結　語

心臓リハビリテーションの中心の一つである運動療法は，組織および血中の炎症を抑制することにより動脈硬化の発症や進展を抑制すること，さらに運動療法と薬物治療を含めた包括的治療が，動脈硬化の発症や進展抑制に極めて有用であることが基礎的検討からも示唆された．

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12 糖尿病と血管合併症

II. 治療
糖尿病の血管合併症予防を目指した集学的リスク管理

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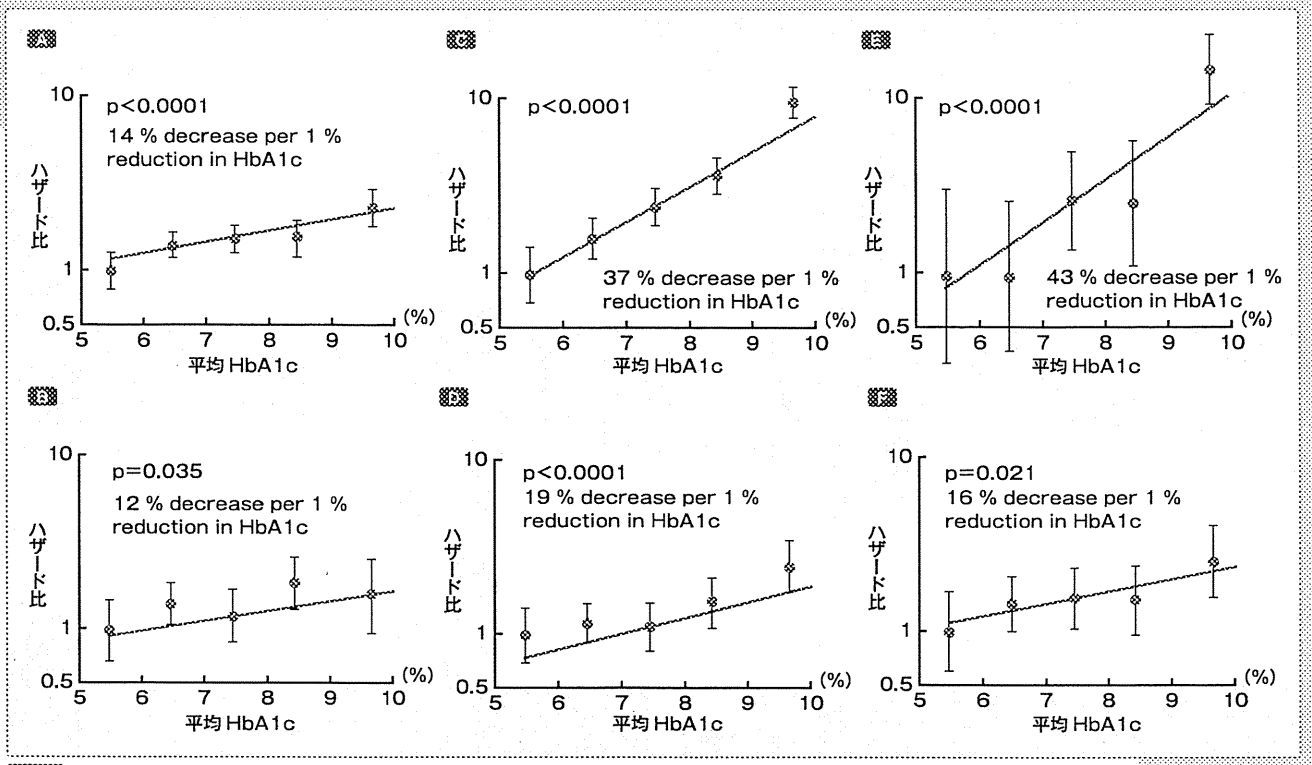
糖尿病は，Framingham Heart Studyを始めとする疫学研究により，心血管疾患において独立した危険因子と認識されるようになった．日本において，糖尿病患者数は年々加速度的に増加しており，その数は約900万人と推計され，耐糖能異常者を合わせると2200万人を超えるといわれている．現在の糖尿病の発症には，生活習慣の欧米化による内臓脂肪の蓄積が強くかかわっている．内臓脂肪の蓄積はインスリン抵抗性を惹起し，その結果メタボリックシンドロームとなる．このように肥満症を背景としたメタボリックシンドロームの増加は，冠危険因子の重積を合併した2型糖尿病患者を増加させている．糖尿病の血管合併症では，網膜症，腎症，神経症の細小血管合併症に加えて，冠動脈疾患や脳血管疾患，末梢動脈疾患の大血管合併症が注目され，糖尿病患者は非糖尿病患者と比較して心血管合併症の発症率は2～4倍になるといわれている．これらの血管合併症の抑制，健康寿命の延長，そして生活の質を維持することが，糖尿病治療へのゴールと考えられる．

現在，日本人糖尿病患者の血管合併症による死亡は約26.8％であり，腎不全を除いた心血管疾患と脳卒中での死亡率は約20％と報告されている[1)]．一方，米国では，糖尿病患者の心血管イベントによる死亡率は50～80％といわれている．このように，最大死因となりうる心血管イベントへの対策が求められており，本稿では，リスクが重積した糖尿病患者に対する血管合併症の予防戦略を概説する．

糖尿病と血管合併症

糖尿病では，高血糖とインスリン抵抗性を背景として，酸化ストレスの亢進や血管内皮機能の障害などにより動脈硬化が促進しやすい状態にある．欧米の報告では，糖尿病患者は冠動脈疾患の既往がなくとも，冠動脈疾患の既往がある非糖尿病患者と同等の心血管イベント発症率であるが[2)]，2型糖尿病患者に対しての厳格な血糖コントロールが心血管疾患や総死亡のリスクを軽減するかについては明確な結論が出ていない．

これまでUnited Kingdom Prospective Diabetes Study（UKPDS）を始めとする大規模臨床試験により，厳格な血糖コントロールが細小血管合併症に対して抑制効果を示すことは繰り返し証明されている．しかし，心血管疾患や脳卒中などの大血管合併症においては，必ずしも厳格な血糖コントロールは抑制効果を示していない．VADT試験



UKPDS 35におけるHbA1cの1%低下による各合併症の発症リスク(文献6改変)
A：致死性/非致死性心筋梗塞／B：致死性/非致死性脳卒中／C：細小血管症／D：白内障摘出術／E：末梢血管疾患による下肢切断あるいは死亡／F：心不全
※縦軸のハザード比は対数表示

やADVANCE試験では，通常療法群よりも厳格な血糖コントロールが大血管合併症を抑制したとは証明されず[3, 4]，ACCORD試験では，厳格な血糖コントロールは，通常療法群と比較してむしろ総死亡率を増加させる結果となった[5]．これには，強化療法群において低血糖や体重増加の問題があり，厳格な血糖コントロールだけでは糖尿病の血管合併症予防は不十分である可能性が示唆された．

一方，UKPDS 35では，HbA1cの上昇に伴って心血管疾患の発症リスクが増加し，HbA1cを1％低下させることで心筋梗塞の発症を14 %，脳卒中の発症を12 %抑制した[6]（■■）．これにより，厳格な血糖コントロールが大血管合併症をある程度は抑制できる可能性が示された．さらに，UKPDS 33終了後に，10年間の追跡調査として行われたUKPDS 80[7]においては，新規糖尿病患者に対して厳格な血糖コントロールを行った強化療法群では，その後通常療法を行ったとしても，長期予後において全死亡，心筋梗塞の発症が抑制される結果となった．また，それと同時に，肥満の糖尿病患者への薬物療法としてメトホルミンが，心血管イベントの抑制に非常に有効であることも示された（■■）．すなわち，糖尿病と診断されて早期に厳格な血糖コントロールを行うことで細小血管合併症の抑制のみならず，大血管合併症の抑制効果が長期持続すると考えられ，現在のlegacy effect（遺産効果）という概念を確立することとなった．また，最近行われた大規模臨床試験であるUKPDS，PROactive，ACCORD，VADT，ADVANCEのメタ解析では，脳卒中では有意差は認められなかったが，冠動脈疾患や心筋梗塞を厳格な血糖コントロールが抑制することが示された[8]．

また，1型糖尿病患者を対象としたDCCT試験でも，インスリン強化療法群が1次予防として細小血管合併症を抑制，2次介入としてその進展を抑制した．さらにDCCTの追跡観察研究として行われたEDICでは，終了するまでの平均17年間の追跡が行われた．心血管イベントは42 %抑制され，MACE（心血管死，非致死的心筋梗塞，脳梗塞）では57 %の抑制を認めた[9]．これはDCCTでの平均6.5年の厳格な血糖コントロールが記憶され，後の血管合併症を抑制することを確認する結果となり，metabolic memoryなどと呼ばれるようになった．これはlegacy

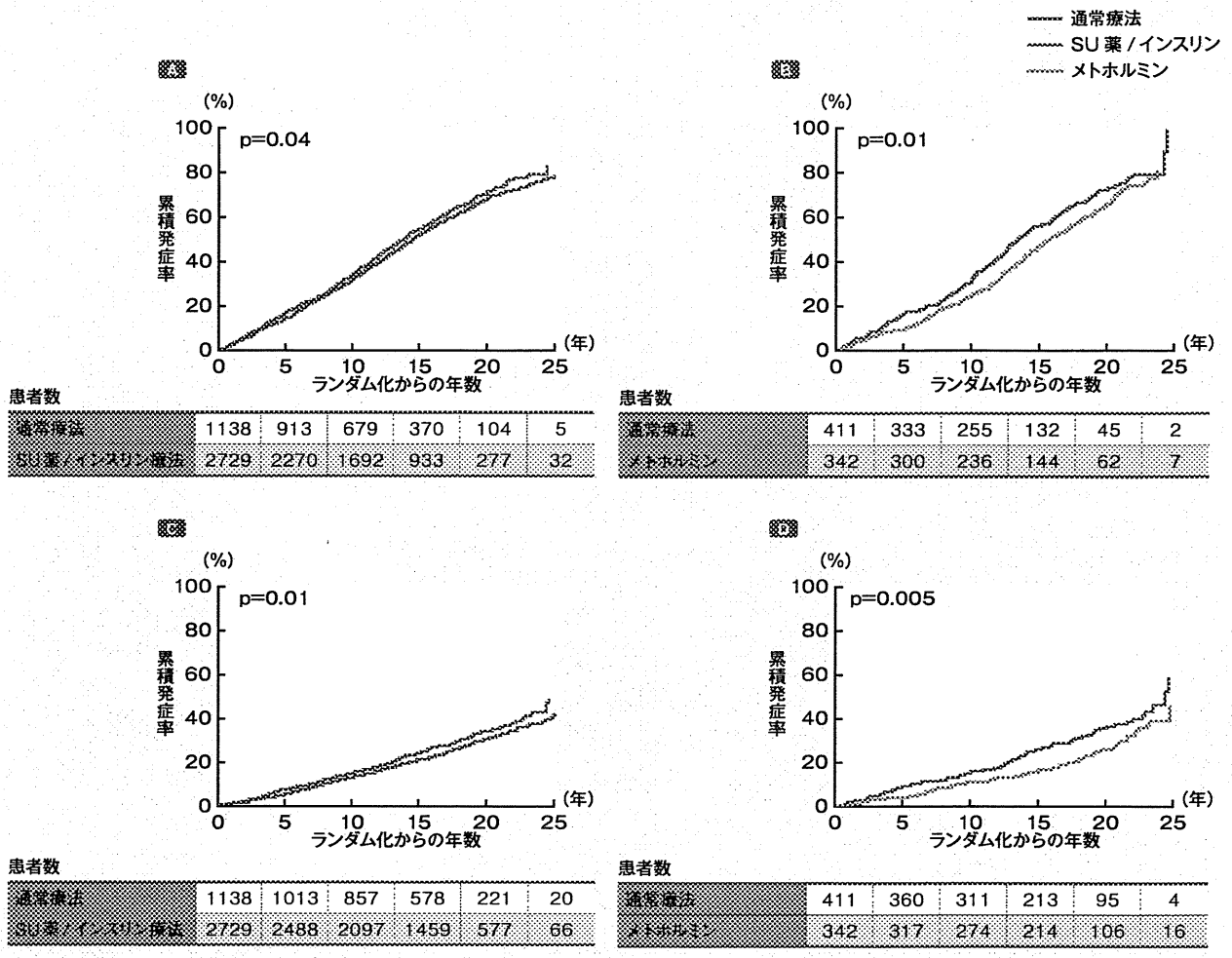


図12 UKPDS 80における legacy effect とメトホルミンによる合併症発症抑制効果(文献7)
A, B：糖尿病関連エンドポイント(A：SU薬/インスリン／B：メトホルミン) ／C, D：心筋梗塞(C：SU薬/インスリン／D：メトホルミン)

effectとほぼ同様の現象と考えられている.

また，治療開始時期も，非常に重要な要素となることが注目されている．糖尿病は高血糖のみではなく，危険因子が複数存在する場合が多いため，早期より動脈硬化が進行するケースが少なくない．実際に，急性心筋梗塞で搬送された患者の70 %が糖尿病，もしくは耐糖能異常であるとの報告もあり[10]，耐糖能異常や空腹時血糖異常である境界型からの介入治療が求められている．日本人を対象としたFunagata Studyでも，食後高血糖が大血管症の重要な危険因子であることが示されており[11]，αグルコシダーゼ阻害薬やグリニド薬により，食後高血糖の改善が血管合併症の抑制につながると期待されている.

薬物療法ついて，SU薬は一般的な血糖降下薬として古くより使用されている．しかし，UGDP研究において，SU薬は潜在的心血管系有害作用を有する可能性が示唆された[12]．以降，とくに心筋虚血プレコンディショニングの概念が生まれてからは，冠動脈疾患の既往がある患者に対してSU薬の使用を懸念する意見もあったが，第3世代SU薬ではその点が改善され，冠動脈への影響はないとされている.

現在は，使用において論議が分かれるところではあるが，チアゾリジン薬による介入を行ったPROactive試験では，投与群において大血管合併症の抑制を認めている[13]．これはチアゾリジン薬の抗炎症硬化やアディポネクチン上昇効果，膵β細胞の保護作用などの多面的な効果が，動脈硬化の進展を抑制しているとの報告もある[14].

現在，インクレチン関連薬として，DPP-4阻害薬やGLP-1

表3 日本人の2型糖尿病患者における心血管疾患，脳卒中の年間発症率と日本人一般住民と糖尿病患者の比較（文献15改変）

		冠動脈疾患	脳卒中
Japan Diabetes Complications Study（JDCS）		9.6	7.5
		男性 11.2	男性 8.5
		女性 7.9	女性 6.6
日本人一般住民モデル（久山町研究）		男性 3.5	男性 5.3
		女性 1.8	女性 3.9
UKPDS	コントロール	17.4	5.0
	治療介入	14.7	5.6

表4 日本人の2型糖尿病患者における心血管疾患，脳卒中の危険因子（JDCS）（文献15改変）

	全体	男性	女性
心血管疾患	LDL-C（p＜0.0001）	LDL-C（p＜0.001）	TG（p＜0.01）
	TG（p＜0.0001）	TG（p＜0.01）	糖尿病罹病期間（p=0.01）
	HbA1c（p=0.04）	喫煙（p=0.02）	
		HbA1c（p=0.04）	
脳卒中	収縮期血圧（p=0.02）	収縮期血圧（p=0.04）	
脳卒中 and/or 心血管疾患	LDL-C（p＜0.0001）	LDL-C（p＜0.0001）	収縮期血圧（p=0.02）
	TG（p＜0.0001）	TG（p＜0.0001）	TG（p＜0.0001）
	収縮期血圧（p=0.02）	喫煙（p=0.02）	
	HbA1c（p=0.04）		
	喫煙（p=0.02）		

受容体作動薬が新しい治療薬として注目されている．GLP-1受容体作動薬は血管内皮機能の改善や膵β細胞のアポトーシス抑制による膵保護作用が報告されており，今後の臨床データによるエビデンスの集積が期待されている．

糖尿病と高血圧症

Japan Diabetes Complications Study（JDCS）の登録患者において，脳卒中の年間発症率は1000人あたり7.5人であり[15]，一般住民モデルとされている久山町研究と比較して約1.5倍の発症率であった（表3）．また，UKPDSのデータと比較して，日本人の糖尿病患者における脳卒中の発症率は高いことが示された．

脳卒中の最大の危険因子は高血圧であるが，JDCS登録患者の最大の危険因子も収縮期血圧である（表4）．高血圧症を合併した2型糖尿病患者を対象に厳格な血圧管理と緩やかな血圧管理を比較したUKPDS 38では，厳格な血圧管理群の血圧は平均144/82 mmHgであり，緩やかな血圧管理群の血圧は平均154/87 mmHgであった．厳格管理（強化療法）群は緩やかな血圧管理（通常療法）群と比較して有意な血圧低下が認められ，脳卒中を44％抑制した[16]（表5）．ACCORD BP試験においても，収縮期血圧120 mmHg未満の群は，140 mmHg未満の集団と比較して脳血管疾患の発症率を抑えた結果となっている[17]．脳卒中の合併を抑制するには，厳格な血圧コントロールが必要不可欠である．

日本高血圧学会の『高血圧ガイドライン2009』によると，日本の糖尿病合併高血圧の降圧目標は130/80 mmHg未満である．Bangaloreらのメタ解析では，耐糖能異常，空腹時血糖異常を含めた2型糖尿病患者において，収縮期血圧135 mmHgまで降圧させると脳卒中の発症率は下がり，130 mmHg未満になるとより顕著に抑制効果が認められた（表6）．降圧目標を達成するための治療が求められる[18]．

薬物治療においては，臓器保護作用やインスリン抵抗性の改善作用を目的としたレニン・アンジオテンシン系抑制薬であるACE阻害薬，あるいはアンジオテンシンⅡ受容体拮抗薬（ARB）を第1選択とし，確実な降圧作用を重視してCa拮抗薬やサイアザイド系利尿薬を第2選択としている．糖尿病患者の高血圧は治療抵抗性であること

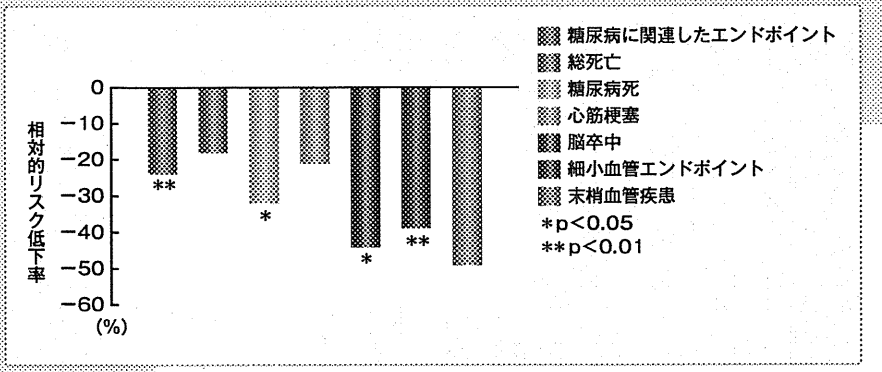


図1 UKPDS 36における厳格な降圧治療による各合併症の抑制効果(文献16改変)
糖尿病に関連したエンドポイント：突然死，心筋梗塞，脳卒中，腎不全，失明など

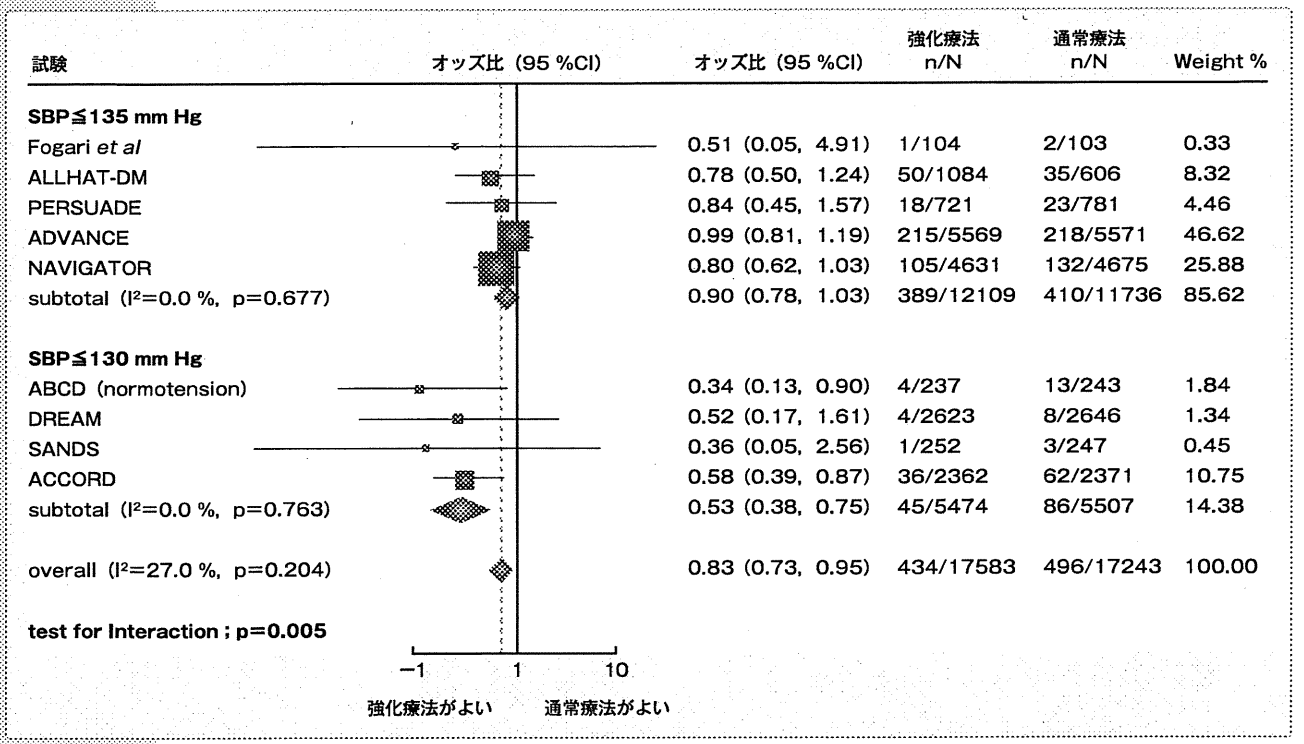


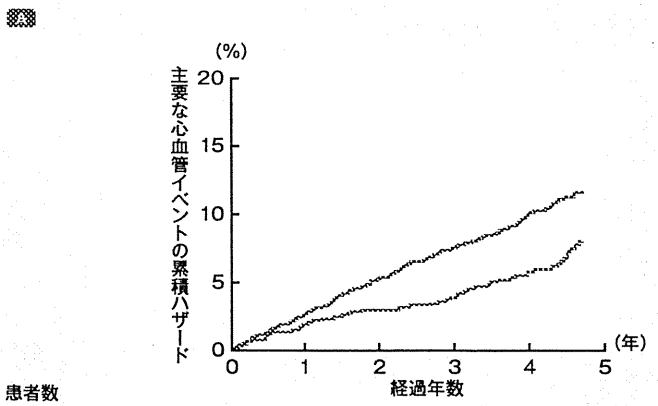
図2 Bangaloreらのメタ解析における血圧コントロールと脳卒中の抑制効果(文献18一部改変)

が多く，複数の降圧薬を併用するケースは少なくない．ACCOMPLISH試験では，ACE阻害薬の併用薬として，利尿薬よりもCa拮抗薬を併用した群において，心血管疾患による死亡・発症が20 %抑制されたと報告している[19]．

糖尿病と脂質異常症

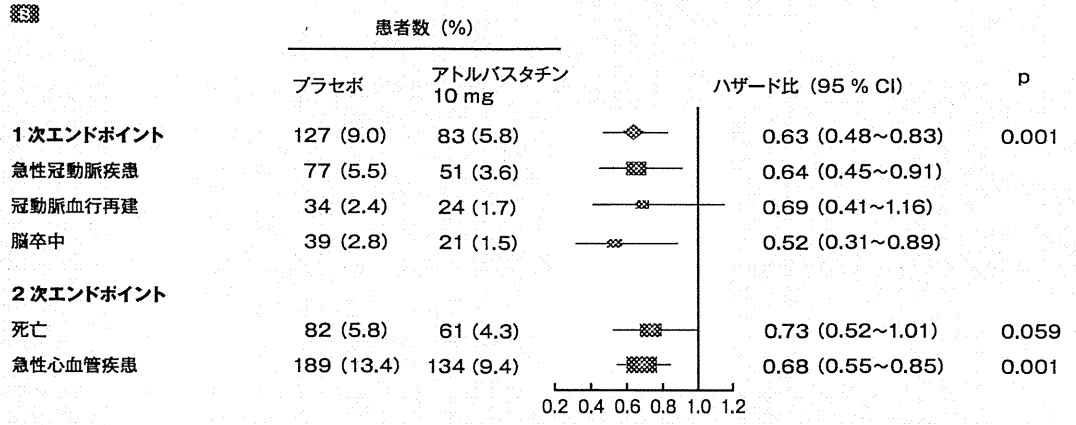
JDCSによると，冠動脈疾患の発症率は1000人あたり年間9.6人であり，脳卒中の発症リスクとほぼ同等かやや高い結果であり，日本において注目されるべき結果である．また，女性と比較して，男性は1.5倍程度高い結果であった．

近年，内臓脂肪蓄積を主体としたメタボリックシンドロームが周知されてきた．メタボリックシンドロームを背景とした糖尿病患者の多くは，脂質異常症や肥満などの他の危険因子を多く持っており，これらは心血管のリスクをさらに上げることが知られている．JDCSの結果では，冠動脈疾患の危険因子としてLDLコレステロールとTGに加えてHbA1cが関与していた．つまり，心血管疾患の合併は脂



患者数

プラセボ	1410	1351	1306	1022	651	305
アトルバスタチン	1428	1392	1361	1074	694	328

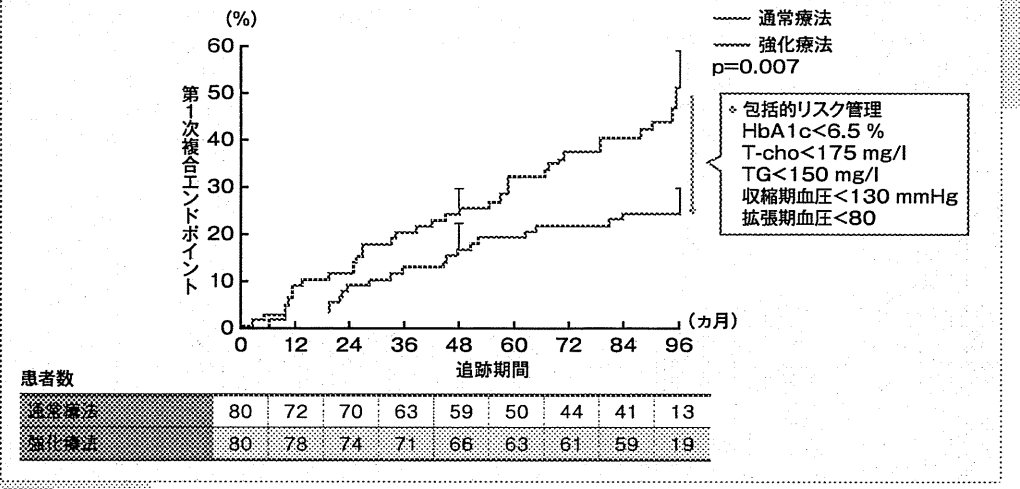


CARDS試験におけるアトルバスタチンの冠動脈疾患・脳卒中の抑制効果(文献21より改変)

質と血糖値の管理が重要なポイントであることが示された.

心血管疾患発症の抑制を行ううえで,スタチン製剤は最もエビデンスを確立した薬剤である.Heart Protection Study (2003) では,糖尿病の有無にかかわらず,スタチンによるLDLコレステロール低下療法が心血管イベントを抑制することが示された[20].糖尿病患者においても,大血管合併症の抑制の脂質介入には,LDLコレステロールの低下を目標に介入することが一般的となった.また,2型糖尿病患者を対象にHMG-CoA還元酵素阻害薬の心血管イベント1次予防効果を検討したCARDS試験では,アトルバスタチン10 mg/日を介入することで,LDLコレステロールを40 %低下させることが示された.プラセボ群と比較して,心筋梗塞の発症を早期より抑制することができ,また,脳卒中の発症も抑制された[21] (図).本来,LDLコレステロールは脳卒中において重大な危険因子とはいえないにもかかわらず抑制効果が得られたことは,スタチンによる脂質治療介入の広範囲の可能性を示したものと考えられる.

また,JDCSでも示されたとおり,LDLコレステロールの次にトリグリセリド (TG) も非常に重要な危険因子であることは,CARDS試験で抑制しきれなかった約60 %の心筋梗塞例を抑制するためのさらなる介入方法となる可能性がある.PERISCOPE試験では,ピオグリタゾン投与群においてTG/HDL比が是正され,プラークの進行抑制効果が得られている[22].LDLコレステロールだけではなく,TGの改善が,イベント抑制にかかわっている可能性もある.TGへの治療として,FIELD研究においては,2型糖尿病患者を対象にフェノフィブラートの有用性を検討した



患者数									
通常療法	80	72	70	63	59	50	44	41	13
強化療法	80	78	74	71	66	63	61	59	19

図1 Steno-2試験における，包括的リスク管理による細小血管合併症・大血管合併症の抑制効果（文献2を改変）

結果，冠動脈イベントでは有意差を示すことはできなかったが，全心血管系イベントではフィブラート投与群が有意にリスクを減少させることが示されている[23]．

おわりに

糖尿病患者において血管合併症を抑制するためには，Steno-2試験[24]で示されたような血糖値・脂質・血圧に加えて，喫煙や肥満などの集積した危険因子に対しての包括的な介入が求められている（図1）．今まで述べたように，薬物療法は非常に重要であるが，運動療法や食事療法を基軸とした生活習慣の改善や肥満を起こさせない健康生活の指導が基本であることを忘れてはならない．現在進行中であるJ-DOIT3では，血糖値，血圧，LDLコレステロールやTGを食事療法と運動療法を重視しており，緩徐に血糖値をコントロールしていく本試験のプロトコールは，低血糖・体重を増加させない安全な治療法を確立するための手助けとなるであろう．

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Effects of prolonged expiration breathing on cardiopulmonary responses during incremental exercise

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ABSTRACT

This study was designed to clarify the effects of breathing with prolonged expiration on cardiopulmonary responses and autonomic nervous activity during incremental exercise. Eleven healthy men were randomly assigned to breathing mode: a prolonged expiration breathing with a 2-s inspired time and 4-s expired time and a spontaneous breathing without any constraints. Oxygen uptake ($\dot{V}_{O_2}$), ventilation efficiency ($\dot{V}_E/\dot{V}_{CO_2}$) and rate pressure product were measured. Low- (LF) and high-frequency (HF) components of blood pressure and heart rate variability were analyzed to assess sympathetic and parasympathetic nervous activities, respectively. $\dot{V}_E/\dot{V}_{CO_2}$, rate pressure product and LF were significantly lower, and $\dot{V}_{O_2}$ and HF were significantly higher during exercise with prolonged expiration than with spontaneous breathing. Striking effects of prolonged expiration breathing included the improvement of ventilation efficiency, the suppression of sympathetic nervous activity and the activation of parasympathetic one during incremental exercise. Furthermore, prolonged expiration breathing may have suppressed the exercise-induced increase in myocardial $\dot{V}_{O_2}$.

1. Introduction

The breathing technique of decreasing respiratory frequency (f_R) and increasing tidal volume (V_T) reportedly suppresses sympathetic nervous activity and activates parasympathetic nervous activity (Bernardi et al., 1998; Goso et al., 2001). Although many studies have demonstrated an interaction between breathing technique and autonomic nervous activity as assessed by heart rate and blood pressure variability (Bartels et al., 2004; Bloomfield et al., 2001; Cottin et al., 1999; Pöyhönen et al., 2004; Sanderson et al., 1996), few have documented the effects of breathing mode on cardiopulmonary responses during exercise. Our prolonged expiration breathing technique, involving 2-s inspired time (T_I) and 4-s expired time (T_E), capitalizes on the suppression of parasympathetic nervous activity during inspiration and its respective activation during expiration (Hayano et al., 1994a,b), which occurs concurrently with suppression of sympathetic nervous activity during expiration (Seals et al., 1993). We previously reported that prolonged expiration breathing enhances parasympathetic nervous activity and suppresses heart rate elevation in healthy young volunteers in comparison to breathing with a 3-s T_I and 3-s T_E or spontaneous breathing during exercise with a moderate workload (Matsumoto et al., 2008). It is known that heart rate and blood pressure are excessively elevated during exercise in patients with acute myocardial infarction or chronic heart failure in whom sympathetic nervous activity is increased and parasympathetic nervous activity is weakened, resulting in decreased exercise tolerance (Goldsmith et al., 2000; Rosenwinkel et al., 2001; Matsunaga et al., 2004). Therefore, we hypothesized that prolonged expiration breathing during exercise would enhance parasympathetic nervous activity and enable safe exercise therapy without excessively elevating heart rate or blood pressure. However, the effects of prolonged expiration breathing on cardiopulmonary responses and autonomic nervous activity as exercise intensity changes are largely unknown. Exercise is prescribed at a variety of intensities for patients with cardiopulmonary disease according to their condition. To develop a clinically applicable exercise program using prolonged expiration breathing, it is necessary to assess how this technique affects cardiopulmonary responses and autonomic nervous activity in increments from low exercise intensity to the maximum exertion.

We hypothesized that prolonged expiration breathing would enhance parasympathetic nervous activity and decrease sympathetic nervous activity during incremental exercise, resulting in the

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Table 1
Participants' charcteristics.

Participants	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m^2)	Peak $\dot{V}_{O_2}$ (mL/min/kg)		Peak work rate (W)	
					Spontaneous breathing	Prolonged expiration	Spontaneous breathing	Prolonged expiration
1	22	176	68	22.0	40.4	43.5	218	210
2	23	182	78	23.5	38.5	45.2	208	230
3	20	168	60	21.3	36.8	38.1	192	202
4	22	185	72	21.0	42.3	42.9	234	238
5	21	175	63	20.6	38.3	38.6	212	214
6	24	171	58	19.8	37.5	37.5	196	196
7	22	165	55	20.2	35.3	36.2	188	194
8	22	169	57	20.0	37.6	38.0	196	204
9	22	174	63	20.8	38.9	41.2	210	216
10	24	180	72	22.2	39.7	43.3	212	226
11	23	173	65	21.7	38.8	39.3	208	214
Means ± SEM	22.1 ± 0.4	174.6 ± 1.9	64.8 ± 2.2	22.1 ± 0.3	38.6 ± 2.0	40.3 ± 2.4†	204.8 ± 6.8	213.6 ± 7.7†

SEM: standard error; BMI: body mass index; $\dot{V}_{O_2}$: oxygen uptake.
† $P < 0.05$ vs. spontaneous breathing.

suppression of excessive heart rate and blood pressure elevation, in comparison to spontaneous breathing in healthy young volunteers. Therefore, this study was designed to investigate the effects of prolonged expiration breathing on cardiopulmonary responses and autonomic nervous activity during incremental exercise in healthy young volunteers.

2. Methods

2.1. Participants

The study participants were 11 healthy young men. Participant characteristics are presented in Table 1. The study protocol was approved by the Ethics Committee of Kitasato University, and informed consent was obtained from each participant after a detailed explanation of the study protocol. All participants volunteered to participate in the study, and none of the volunteers received monetary compensation. Participants were excluded if they had a history of smoking, cardiopulmonary disease or motor dysfunction due to orthopedic or central neurological disease. The participants were instructed to maintain their usual sleep patterns, refrain from exercise, and abstain from caffeine and other autonomic stimulants for 2 days before the study. The study was performed between 14:00 and 17:00 in an air-conditioned room kept at 23–25 °C and 40–60% humidity.

2.2. Study design

This study employed a randomized, cross-over design. Participants were randomly assigned to groups performing controlled breathing with prolonged expiration first or spontaneous breathing first. For prolonged expiration breathing, they were instructed to breathe with a 2-s T_I and 4-s T_E at 10 breaths/min from the lightest to the heaviest possible workload the incremental exercise progression. The participants were instructed to watch the T_I, T_E and T_E/T_I displayed on a gas analyzer monitor (AE300S, Minato, Osaka, Japan). In addition, they maintained the breathing at approximately a 1:2 ratio of T_I to T_E until the endpoint of incremental exercise, even if they experienced difficulty maintain a 2-s T_I and 4-s T_E. The participants practiced prolonged expiration breathing several times before the study and were supervised by an instructor during incremental exercise. For the spontaneous breathing, they were instructed to breathe without constraint throughout incremental exercise. The incremental exercises with prolonged expiration breathing or spontaneous breathing were performed at the same time on 2 consecutive days.

2.3. Study protocol

Incremental symptom-limited cardiopulmonary exercise testing was performed using a recumbent cycle ergometer (Strength-Ergo.240, Mitsubishi Electric Engineering, Tokyo, Japan) according to a ramp protocol. After resting for 15 min on the recumbent cycle ergometer, participants started exercise at 10 watts (W) and 50 revolutions per minute of pedaling for 3 min as a warm-up period. Next, the exercise was performed with a workload increase of 1 W every 3 s (20 W/min) until the endpoint of cardiopulmonary exercise testing, followed by a 3-min cool-down period at 10 W and finally a 5-min recovery period. The endpoint of cardiopulmonary exercise testing was determined according to the criteria of the American College of Sports Medicine (Kelsey, 2001). That is, the cardiopulmonary exercise testing was finished when the participants could not continue pedaling at 50 revolutions or more per minute, or when they were physically exhausted or complained of severe dyspnoea or dizziness.

2.4. Cardiopulmonary responses

The T_I, T_E, T_E/T_I, f_R, V_T, expired ventilation ($\dot{V}_E$), oxygen uptake ($\dot{V}_{O_2}$), carbon dioxide output ($\dot{V}_{CO_2}$), and ventilatory equivalents for $\dot{V}_{O_2}$($\dot{V}_E/\dot{V}_{O_2}$) and $\dot{V}_{CO_2}$($\dot{V}_E/\dot{V}_{CO_2}$) were measured with a breath-by-breath method using a gas analyzer throughout the study. The average values were computed every 10 s. $\dot{V}_{O_2}$ and work rate were used as parameters of aerobic capacity (Wasserman et al., 2005). The anaerobic threshold was calculated from $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ using the *V*-slope method (Koike et al., 2002). The ratio of $\dot{V}_E$ increase to $\dot{V}_{CO_2}$ increase ($\dot{V}_E/\dot{V}_{CO_2}$ slope) was calculated by least-squares linear regression using $\dot{V}_E$ and $\dot{V}_{CO_2}$ measured from the beginning of incremental exercise to the respiratory compensation point (Wasserman et al., 2005). The $\dot{V}_E/\dot{V}_{O_2}$, $\dot{V}_E/\dot{V}_{CO_2}$ and $\dot{V}_E/\dot{V}_{CO_2}$ slope were used as parameters of ventilation efficiency (Koike et al., 2002; Wasserman et al., 2005). The delta $\dot{V}_{O_2}$/delta work rate was calculated by least-squares linear regression using the delta $\dot{V}_{O_2}$ and delta work rate measured from 1 min after the beginning of incremental exercise to the anaerobic threshold (Toyofuku et al., 2003). The $\dot{V}_{O_2}$/work rate and delta $\dot{V}_{O_2}$/delta work rate were used as parameters of exercise efficiency (Wasserman et al., 2005). Percutaneous oxygen saturation (Sp_{O_2}) was continuously monitored using a pulse oximeter (PULSOX®-Me, Nihon Kohden, Tokyo, Japan) throughout the study.

The heart rate, and systolic and diastolic blood pressures were recorded continuously throughout the study using a Holter electrocardiogram (FM-120, Fukuda Denshi, Tokyo, Japan) and a finger sphygmomanometer (Finometer, Finapres Medical System BV, The

Netherlands), respectively. The rate pressure product was calculated from heart rate multiplied by systolic blood pressure as a parameter reflecting myocardial $\dot{V}_{O_2}$ (Nelson et al., 1974; Gobel et al., 1978).

2.5. Heart rate variability and blood pressure variability

Offline beat-to-beat analyses of the digitized Holter electrocardiogram and Finometer signals were performed at a temporal resolution of 1 ms. Time series of successive beats were extracted for R–R intervals and beat-to-beat intervals. Occasional ectopic beats were corrected by linear interpolation of adjacent normal beats (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Any significant trends were removed by subtraction of the best polynomial function fitted to the data using low-pass filtering. Heart rate and blood pressure variability were analyzed by the maximum entropy method using MemCalc (MemCalc/TARAWA, GMS, Tokyo, Japan) to obtain the power spectra of the low-frequency (LF; 0.04–0.15 Hz) and high-frequency (HF; 0.15–0.4 Hz) components. The power spectrum of the HF component of heart rate variability is used as a parameter reflecting cardiac parasympathetic nervous activity (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996), while that of the LF component of blood pressure variability indicates vascular sympathetic nervous activity (Baselli et al., 1986; Laitinen et al., 1999). The LF/HF ratio in heart rate variability indicates the predominance of sympathetic nervous activity (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). The LF, HF and LF/HF ratio in heart rate and blood pressure variability were analyzed at 10-s intervals for this study protocol.

2.6. Rating of perceived exertion

The rating of perceived exertion was assessed every minute throughout the study according to the Borg scale (Noble et al., 1983).

2.7. Statistical analysis

All resting and warm-up parameters are shown as mean values measured during the 15-min resting period and 3-min warm-up period, respectively. The parameters measured at rest were adopted as baseline values. The f_R, V_T, $\dot{V}_E$, T_E, T_E/T_I and Sp_{O_2} measured during incremental exercise are shown as mean values calculated every quarter of the individual's particular exercise time period from initiation to endpoint. The other parameters measured during incremental exercise are shown as mean values calculated every tenth of the individual's particular exercise time period from initiation to endpoint. Two-way analysis of variance for repeated measures (3 time courses of rest, warm-up and exercise vs. 2 breathing techniques) were used to analyze differences in cardiopulmonary responses and autonomic nervous activity between the prolonged expiration and spontaneous breathing modes. If an F ratio was significant, differences in cardiopulmonary responses and autonomic nervous activity between the prolonged expiration and spontaneous breathing modes were assessed using the paired t-test. All values were expressed as means ± standard error and a P value less than 0.05 was considered statistically significant. Descriptive analyses were performed using SPSS 11.0 J software for Windows (SPSS Japan Inc., Tokyo, Japan). The abbreviations used in the present study are shown in Table 2.

Table 2
Abbreviations.

LF: low-frequency component of the power spectrum
HF: high-frequency component of the power spectrum
T_I: inspired time
T_E: expired time
f_R: respiratory frequency
V_T: tidal volume
$\dot{V}_E$: expired ventilation
$\dot{V}_{O_2}$: oxygen uptake
$\dot{V}_{CO_2}$: carbon dioxide output
Sp_{O_2}: percutaneous oxygen saturation

3. Results

3.1. Exercise times and work rate

All participants completed protocols without any adverse events or complications, and the reason for exercise stoppage was leg fatigue. The exercise times from the beginning of incremental exercise until the anaerobic threshold and endpoint were 6 min 12 ± 32 s and 10 min 2 ± 13 s with prolonged expiration breathing, and 5 min 56 ± 19 s and 9 min 42 ± 24 s with spontaneous breathing, respectively. The work rate at the anaerobic threshold and endpoint were 126.8 ± 10.6 and 213.6 ± 7.7 W with prolonged expiration breathing, and 109.6 ± 10.5 and 204.8 ± 6.8 W with spontaneous breathing, respectively. The exercise times and work rate at the anaerobic threshold and endpoint were significantly higher with prolonged expiration breathing than spontaneous breathing ($P < 0.05$).

3.2. Respiratory responses

Changes in respiratory responses during incremental exercise are shown in Table 3. The f_R was significantly lower from the warm-up to the 75% point of the exercise period with prolonged expiration breathing than with spontaneous breathing ($P < 0.01$). The V_T was significantly higher from the warm-up to the 75% point of the exercise period with prolonged expiration breathing than with spontaneous breathing ($P < 0.01$). From the 50% point to the 75% point of the exercise period, $\dot{V}_E$ was significantly lower with prolonged expiration breathing than with spontaneous breathing ($P < 0.01$).

The T_E was significantly higher from the warm-up to the 75% point of the exercise period with prolonged expiration breathing than with spontaneous breathing ($P < 0.01$). The T_E/T_I was significantly higher in the warm-up and exercise periods with prolonged expiration breathing than with spontaneous breathing ($P < 0.01$).

There was no significant difference in Sp_{O_2} throughout the study between the prolonged expiration and spontaneous breathing modes.

Changes in $\dot{V}_E/\dot{V}_{O_2}$ and $\dot{V}_E/\dot{V}_{CO_2}$ during incremental exercise are shown in Fig. 1. The $\dot{V}_E/\dot{V}_{O_2}$ was significantly lower from the 60% point to the 90% point of the exercise period with prolonged expiration breathing than with spontaneous breathing ($P < 0.01$). The $\dot{V}_E/\dot{V}_{CO_2}$ was significantly lower from the 10% point to the 80% point of the exercise period with prolonged expiration breathing than with spontaneous breathing ($P < 0.01$). The $\dot{V}_E/\dot{V}_{CO_2}$ slope was 19.1 ± 2.9 with prolonged expiration breathing and 22.1 ± 4.4 with spontaneous breathing, and this difference was statistically significant ($P < 0.05$).

3.3. Cardiopulmonary responses

Changes in heart rate, blood pressure and rate pressure product during incremental exercise are shown in Fig. 2. There was no

Table 3
Changes in respiratory responses during incremental exercise.

	Breathing mode	Rest	Warm-up	Incremental exercise			
				25%	50%	75%	100% (endpoint)
f_R (breath/min)	Spontaneous breathing	18.1 (0.8)	24.0 (2.8)	22.7 (2.3)	24.7 (1.6)	27.9 (2.1)	35.8 (2.6)
	Prolonged expiration	17.3 (1.2)	10.1 (0.1)††	10.0 (0.2)††	11.1 (0.6)††	13.4 (3.4)††	29.0 (4.9)
V_T (L)	Spontaneous breathing	0.6 (0.0)	0.7 (0.0)	1.0 (0.0)	1.2 (0.0)	1.5 (0.0)	1.7 (0.0)
	Prolonged expiration	0.6 (0.0)	1.8 (0.2)††	2.0 (0.3)††	2.5 (0.2)††	2.8 (0.2)††	2.4 (0.4)
$\dot{V}_E$ (L/min)	Spontaneous breathing	9.9 (0.3)	16.6 (0.7)	21.4 (1.6)	30.4 (1.6)	44.1 (3.1)	62.9 (5.7)
	Prolonged expiration	9.1 (0.5)	18.3 (2.2)	19.7 (2.1)	25.1 (1.5)††	33.8 (2.5)††	58.1 (6.9)
T_E (s)	Spontaneous breathing	2.1 (0.1)	1.6 (0.1)	1.6 (0.2)	1.4 (0.1)	1.2 (0.1)	0.9 (0.1)
	Prolonged expiration	2.2 (0.1)	3.9 (0.1)††	4.0 (0.1)††	3.7 (0.1)††	3.7 (0.3)††	1.7 (0.4)
T_E/T_I	Spontaneous breathing	1.5 (0.1)	1.4 (0.1)	1.3 (0.0)	1.2 (0.1)	1.2 (0.0)	1.1 (0.0)
	Prolonged expiration	1.4 (0.1)	1.9 (0.1)††	1.9 (0.2)††	1.9 (0.1)††	2.0 (0.1)††	1.9 (0.1)††
Sp_{O_2}	Spontaneous breathing	98.2 (0.4)	98.2 (0.4)	98.3 (0.4)	98.2 (0.4)	97.6 (0.4)	96.8 (0.4)
	Prolonged expiration	98.6 (0.2)	98.6 (0.2)	98.4 (0.3)	98.4 (0.2)	97.9 (0.4)	96.8 (0.4)

Data are presented as means (±standard error). f_R: respiratory frequency; V_T: tidal volume; $\dot{V}_E$: expired ventilation; T_E: expired time; T_I: inspired time; Sp_{O_2}: percutaneous oxygen saturation. The results of the two-way analysis of variance for repeated measures were as follows: interaction between time courses and breathing modes for f_R ($F=2.2$, $P<0.05$), V_T ($F=2.3$, $P<0.05$), T_E ($F=7.8$, $P<0.01$) and T_E/T_I ($F=3.1$, $P<0.01$), and effects of time courses and breathing modes for $\dot{V}_E$ (time courses: $F=51.5$, $P<0.01$, and breathing modes: $F=14.4$, $P<0.01$).
†† $P<0.01$ vs. spontaneous breathing.

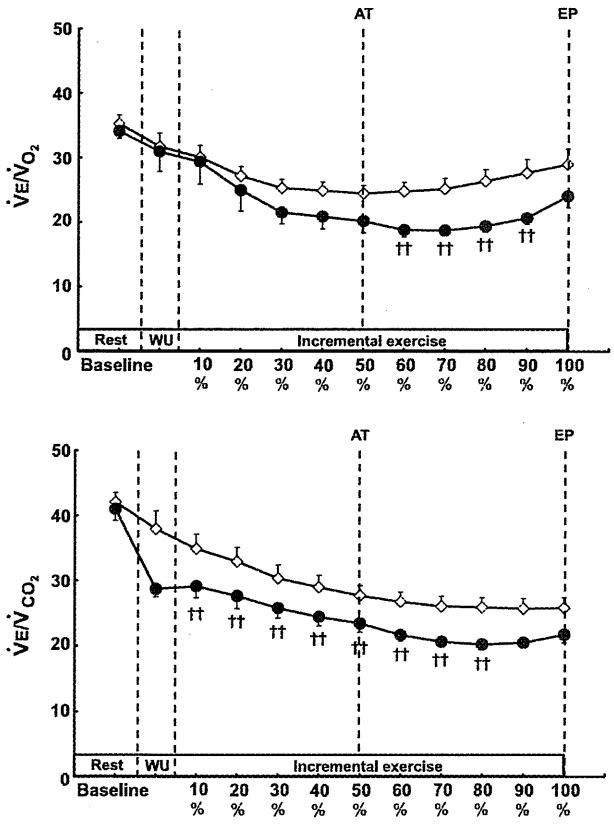

Fig. 1. Changes in $\dot{V}_E/\dot{V}_{O_2}$ and $\dot{V}_E/\dot{V}_{CO_2}$ during incremental exercise. Data are presented as means ± standard error, closed circle: prolonged expiration breathing, open diamond: spontaneous breathing, ††$P<0.01$ vs. spontaneous breathing. $\dot{V}_E$: expired respiration; $\dot{V}_{O_2}$: oxygen uptake; $\dot{V}_{CO_2}$: carbon dioxide emission; WU: warm-up; AT: anaerobic threshold; EP: endpoint. The results of the two-way analysis of variance for repeated measures were as follows: effects of time courses and breathing modes for $\dot{V}_E/\dot{V}_{O_2}$ (time courses: $F=10.0$, $P<0.01$, and breathing modes: $F=27.4$, $P<0.01$) and $\dot{V}_E/\dot{V}_{CO_2}$ (time courses: $F=24.0$, $P<0.01$, and breathing modes: $F=58.2$, $P<0.01$).

significant difference in heart rate throughout the study between prolonged expiration and spontaneous breathing modes. The systolic blood pressure was significantly lower from the 20% point of the exercise period to the endpoint with prolonged expiration breathing than with spontaneous breathing ($P<0.01$). The diastolic blood pressure was significantly lower from the 30% point of the exercise period to the endpoint with prolonged expiration breathing than with spontaneous breathing ($P<0.05$). The rate pressure product was significantly lower from the 20% point of the exercise period to the endpoint with prolonged expiration breathing than with spontaneous breathing ($P<0.01$).

Changes in $\dot{V}_{O_2}$ and $\dot{V}_{O_2}$/work rate during incremental exercise are shown in Fig. 3. The $\dot{V}_{O_2}$ at the endpoint were significantly higher with prolonged expiration breathing than with spontaneous breathing ($P<0.05$). The anaerobic threshold corresponded to 50.9 ± 8.3% of the exercise period with prolonged expiration breathing, and 50.9 ± 2.9% of that with spontaneous breathing, showing no significant difference between breathing modes. The respiratory compensation point occurred at 80.6 ± 8.2% of the exercise period with prolonged expiration breathing and 78.5 ± 6.3% with spontaneous breathing, showing no significant difference between breathing modes. The $\dot{V}_{O_2}$/work rate was significantly higher from the 70% point of the exercise period to the endpoint with prolonged expiration breathing than with spontaneous breathing ($P<0.05$). The delta $\dot{V}_{O_2}$/delta work rate was 10.5 ± 0.4 mL/min/W with prolonged expiration breathing and 9.4 ± 0.5 mL/min/W with spontaneous breathing, and the difference between the two breathing modes was statistically significant ($P<0.05$).

3.4. Heart rate variability and blood pressure variability

Changes in LF, HF and LF/HF of heart rate variability during incremental exercise are shown in Fig. 4. There was no significant differences in LF throughout the study between prolonged expiration and spontaneous breathing modes. The HF was significantly higher from the warm-up to the 50% point of the exercise period with prolonged expiration breathing than with spontaneous breathing ($P<0.01$). The LF/HF was significantly lower from the warm-up to the 20% point of the exercise period with prolonged expiration breathing than with spontaneous breathing ($P<0.05$).

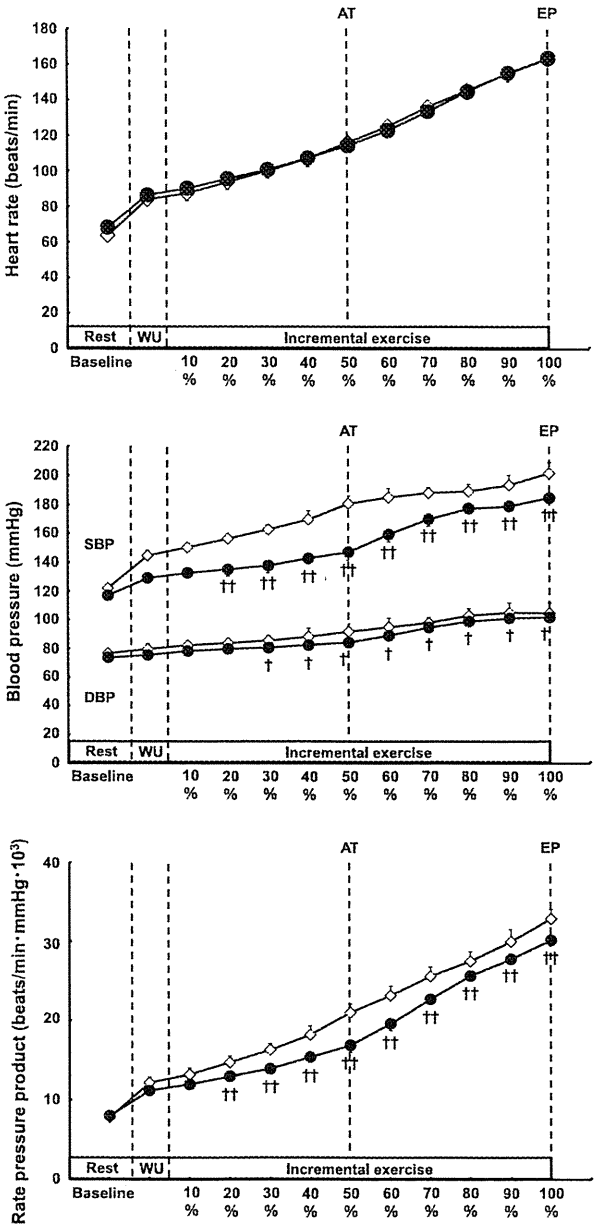

Fig. 2. Changes in heart rate, blood pressure and rate pressure product during incremental exercise. Data are presented as means ± standard error, closed circle: prolonged expiration breathing, open diamond: spontaneous breathing, †$P<0.05$ and ††$P<0.01$ vs. spontaneous breathing. SBP: systolic blood pressure; DBP: diastolic blood pressure; WU: warm-up; AT: anaerobic threshold; EP: endpoint. The results of the two-way analysis of variance for repeated measures were as follows: effect of time courses for heart rate ($F=147.6$, $P<0.01$), and effects of time courses and breathing modes for SBP (time courses: $F=50.7$, $P<0.01$, and breathing modes: $F=110.5$, $P<0.01$), DBP (time courses: $F=53.2$, $P<0.01$, and breathing modes: $F=31.7$, $P<0.01$) and rate pressure product (time courses: $F=154.8$, $P<0.01$, and breathing modes: $F=40.8$, $P<0.01$).

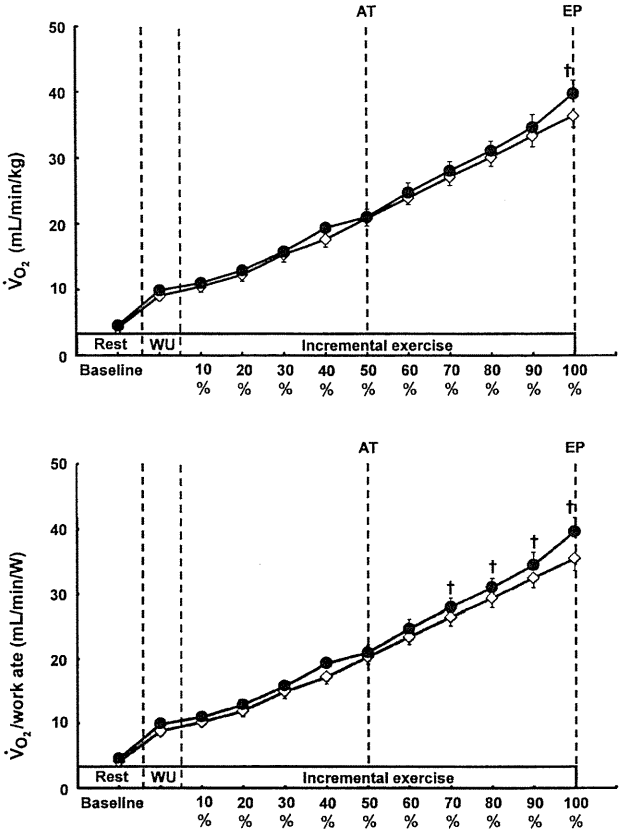

Fig. 3. Changes in $\dot{V}_{O_2}$ and $\dot{V}_{O_2}$/work rate during incremental exercise. Data are presented as means ± standard error, closed circle: prolonged expiration breathing, open diamond: spontaneous breathing, †$P<0.05$ vs. spontaneous breathing. $\dot{V}_{O_2}$: oxygen uptake; WU: warm-up; AT: anaerobic threshold; EP: endpoint. The results of the two-way analysis of variance for repeated measures were as follows: effects of time courses and breathing modes for $\dot{V}_{O_2}$ (time courses: $F=157.2$, $P<0.01$, and breathing modes: $F=4.4$, $P<0.01$) and $\dot{V}_{O_2}$/work rate (time courses: $F=156.5$, $P<0.01$, and breathing modes: $F=9.3$, $P<0.01$).

Changes in LF, HF and LF/HF of blood pressure variability during incremental exercise are shown in Fig. 5. The LF was significantly lower from the 50% point of the exercise period to the endpoint with prolonged expiration breathing than with spontaneous breathing ($P<0.05$ and $P<0.01$, respectively). There was no significant differences in HF throughout the study between prolonged expiration and spontaneous breathing modes. The LF/HF was significantly lower from the 80% point of the exercise period to the endpoint with prolonged expiration breathing than with spontaneous breathing ($P<0.05$).

3.5. Rating of perceive exertion

The respective ratings of perceived exertion at the anaerobic threshold and endpoint were 11.4 ± 0.7 and 16.6 ± 0.7 with prolonged expiration breathing, and 11.2 ± 0.8 and 16.8 ± 0.7 with spontaneous breathing. There were no significant differences in rating of perceived exertion at the anaerobic threshold or endpoint between the prolonged expiration and spontaneous breathing modes.

4. Discussion

The major findings of this study are that prolonged expiration breathing improves ventilation efficiency, suppresses sympathetic nervous activity and activates parasympathetic nervous activity during incremental exercise. Furthermore, prolonged expiration breathing may suppress exercise-induced increases in myocardial $\dot{V}_{O_2}$. Because no significant differences were observed in Sp_{O_2} during exercise between the two breathing modes, we believe that hypoxemia was not induced by the prolonged expiration breathing technique used in this study.

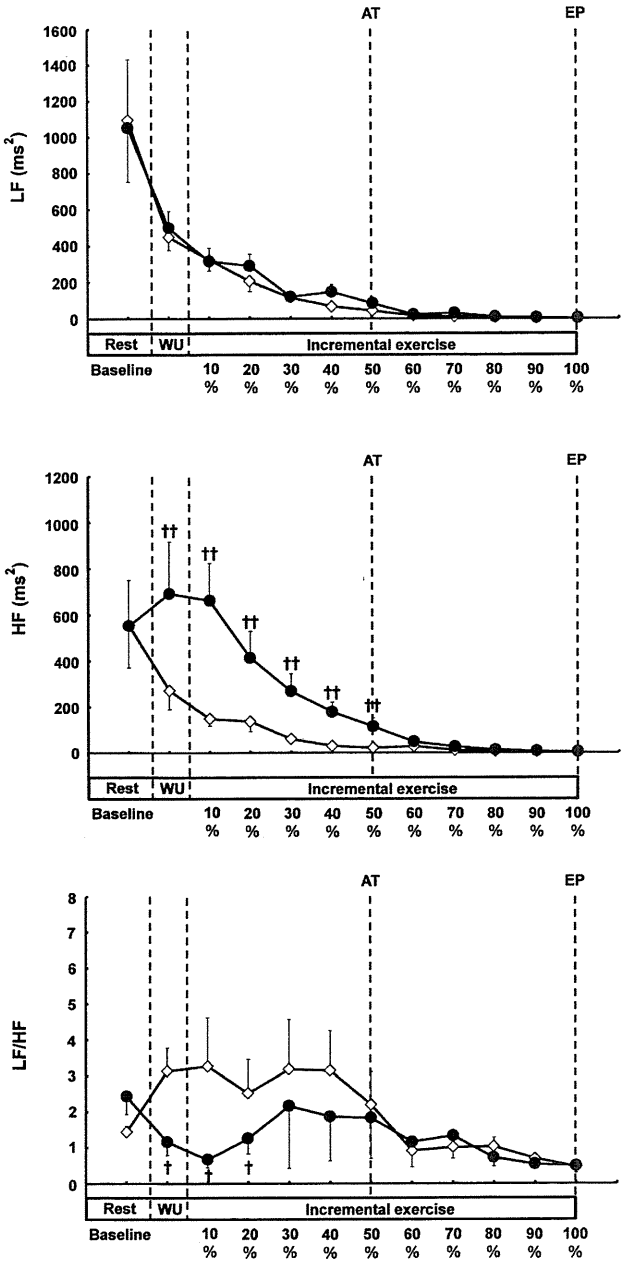

Fig. 4. Changes in LF, HF and LF/HF of heart rate variability during incremental exercise. Data are presented as means ± standard error, closed circle: prolonged expiration breathing, open diamond: spontaneous breathing, $^{\dagger}P<0.05$ and $^{\dagger\dagger}P<0.01$ vs. spontaneous breathing. LF: low-frequency component; HF: high-frequency component; WU: warm-up; AT: anaerobic threshold; EP: endpoint. The results of the two-way analysis of variance for repeated measures were as follows: effect of time courses for LF ($F=15.8$, $P<0.01$) and LF/HF ($F=2.0$, $P<0.05$), and interaction between time courses and breathing modes for HF ($F=2.1$, $P<0.05$).

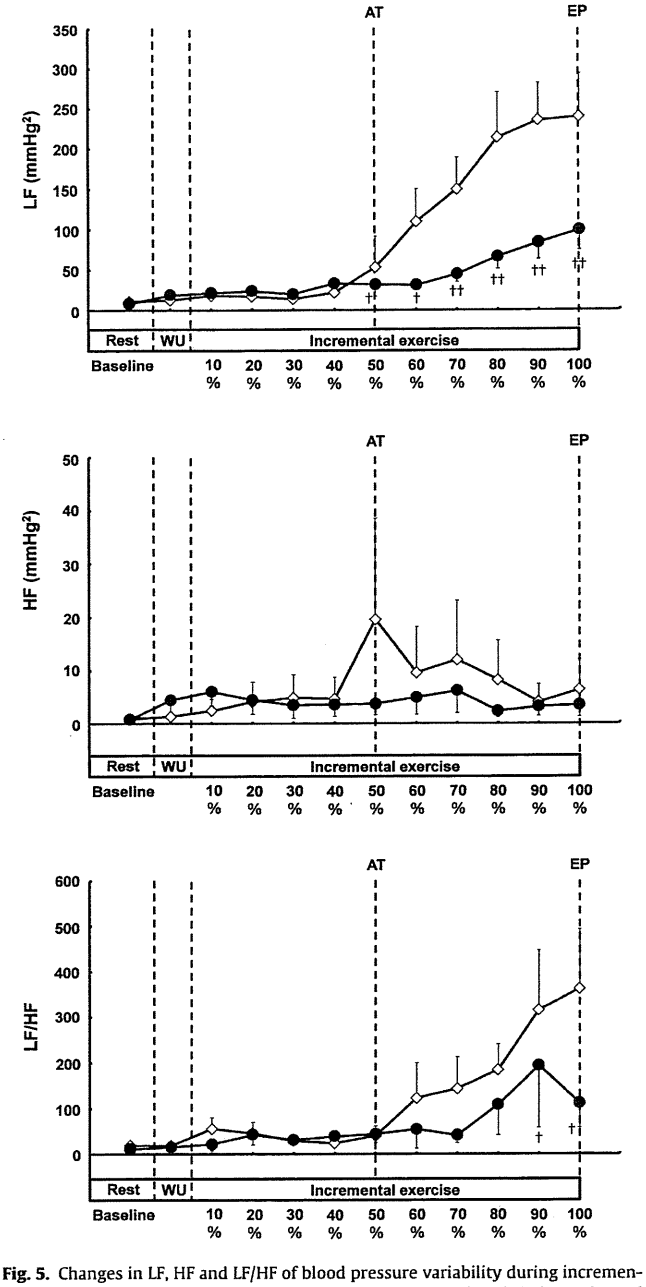

Fig. 5. Changes in LF, HF and LF/HF of blood pressure variability during incremental exercise. Data are presented as means ± standard error, closed circle: prolonged expiration breathing, open diamond: spontaneous breathing, $^{\dagger}P<0.05$ and $^{\dagger\dagger}P<0.01$ vs. spontaneous breathing mode. LF: low-frequency component; HF: high-frequency component; WU: warm-up; AT: anaerobic threshold; EP: endpoint. The results of the two-way analysis of variance for repeated measures were as follows: interaction between time courses and breathing modes for LF ($F=3.7$, $P<0.01$), and effects of time courses and breathing modes for LF/HF (time courses: $F=4.2$, $P<0.05$, and breathing modes: $F=5.3$, $P<0.05$).

With prolonged expiration breathing, the participants were able to control their respiration at 10 breaths/min from the warm-up to the 75% point of the exercise period and to maintain a T_E/T_I of approximately 2 from the warm-up until the end of the to the incremental exercise. It is generally known that $\dot{V}_E$ rises as V_T increases with exercise intensity below anaerobic threshold during incremental exercise, and the $\dot{V}_E$ increase depends on an increased f_R when exercise intensity exceeds anaerobic threshold (Jones and Doust, 1998; Neder et al., 2001, 2003). The present results demonstrate that the $\dot{V}_E$ increase observed with prolonged expiration breathing was controlled by an increase in V_T until the duration of exercise reached the 75% point of the exercise period beyond the time of anaerobic threshold. Furthermore, prolonged expiration breathing was shown to provide more efficient ventilation, due to the lower $\dot{V}_E$, $\dot{V}_E/\dot{V}_{O_2}$, $\dot{V}_E/\dot{V}_{CO_2}$ and $\dot{V}_E/\dot{V}_{CO_2}$ slope, improved oxygen uptake and increased exercise efficiency due to higher $\dot{V}_{O_2}$ and delta $\dot{V}_{O_2}$/delta work rate, as compared with spontaneous breathing mode. The improved ventilation efficiency may be attributable

to the decreased f_R and the increased V_T, which reportedly diminish dead space ventilation and increase alveolar ventilation (Giardino et al., 2003; Thin et al., 2004). Furthermore, a significant negative correlation has already been documented between peak $\dot{V}_{O_2}$ and $\dot{V}_E/\dot{V}_{CO_2}$ slope during exercise (Chua et al., 1997; Clark et al., 1997). The present findings indicate that prolonged expiration breathing, which decreases f_R and increases V_T, improves the efficiency of both ventilation and exercise. However, the $\dot{V}_{O_2}$/work rate with prolonged expiration breathing was higher at high intensities of 70% or above during incremental exercise as compared to that with spontaneous breathing. Although exercise efficiency was diminished at high intensity by prolonged expiration breathing as compared to spontaneous breathing, the $\dot{V}_{O_2}$/work rate might have been higher with prolonged expiration breathing due to improved uptake of $\dot{V}_{O_2}$ at high intensity. Nevertheless, $\dot{V}_{O_2}$ and the work rate at high intensity were high with prolonged expiration breathing because $\dot{V}_E/\dot{V}_{O_2}$, $\dot{V}_E/\dot{V}_{CO_2}$ and $\dot{V}_E/\dot{V}_{CO_2}$ slope, indices of ventilation efficiency, were improved by prolonged expiration breathing as compared to spontaneous breathing. The $\dot{V}_{O_2}$ and work rate, indices of aerobic capacity, appeared to improve with prolonged expiration breathing as compared to spontaneous breathing.

We examined cardiovascular responses with prolonged expiration breathing and found that blood pressure and rate pressure product were significantly lower during exercise period with prolonged expiration breathing than with spontaneous breathing. However, heart rate did not differ significantly between the two breathing modes. Therefore, prolonged expiration breathing was shown to suppress excessive elevations of blood pressure and rate pressure product during incremental exercise, and it might have suppressed the increase in myocardial $\dot{V}_{O_2}$. Our analysis of autonomic nervous activity revealed that HF in heart rate variability was higher and LF in blood pressure variability was lower during incremental exercise with prolonged expiration breathing than with spontaneous breathing. Therefore, we conclude that prolonged expiration breathing contributes to decreases in blood pressure and rate pressure product via the activation of parasympathetic nervous activity and the suppression of sympathetic nervous activity. Conversely, LF/HF in heart rate and blood pressure variability were significantly lower during exercise with prolonged expiration breathing than with spontaneous breathing, although LF in heart rate variability and HF in blood pressure variability did not differ significantly between the two breathing modes. Our findings suggest that prolonged expiration breathing may improve autonomic balance during incremental exercise as compared to spontaneous breathing (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Baselli et al., 1986; Laitinen et al., 1999).

Parasympathetic nervous activity is known to be suppressed soon after the initiation of incremental exercise, whereas sympathetic nervous activity shows no significant change at an exercise intensity below anaerobic threshold (Goldsmith et al., 2000; Rosenwinkel et al., 2001). When exercise intensity exceeds anaerobic threshold, parasympathetic nervous activity decreases markedly while sympathetic nervous activity begins to increase according to the workload increment (Goldsmith et al., 2000; Rosenwinkel et al., 2001). However, our results demonstrate that prolonged expiration breathing suppresses both the decrease of parasympathetic nervous activity after initiation of incremental exercise and the increase of sympathetic nervous activity at exercise intensity exceeding anaerobic threshold. Autonomic nervous activity is known to be synchronized with the central respiratory drive (Barman and Gebber, 1976; Preiss et al., 1975; Spyer, 1990). Thus, parasympathetic nervous activity decreases during inspiration and increases during expiration (Katona and Jih, 1975). In contrast, sympathetic nervous activity decreases during expiration (Seals et al., 1993). The f_R decrease and V_T increase also reportedly activate parasympathetic nervous activity (Hayano et al., 1994a,b) and suppress sympathetic nervous activity (Hayano et al., 1994a,b). Therefore, prolonged expiration breathing, which involves a decrease of f_R and an increase of V_T, appears to effectively improve the balance between parasympathetic and sympathetic nervous activities (Berntson et al., 1993; Daly, 1985; Yasuma and Hayano, 2004).

Ventilation efficiency was improved and aerobic capacity was enhanced by incorporating prolonged expiration breathing, which was examined in this study, during incremental exercise. Prolonged expiration breathing also suppressed excessive increases in blood pressure by activating parasympathetic nervous activity and inhibiting sympathetic nervous activity during incremental exercise. Therefore, exercise with prolonged expiration breathing may be safer and more effective, not only in healthy young adults but also in older individuals, than similar activity with spontaneous breathing. The effects of prolonged expiration breathing on autonomic nervous activity and cardiopulmonary responses in elderly subjects will be elucidated in future studies.

The present study employed frequency components, which are common markers of heart rate variability and blood pressure variability, in the analysis of autonomic nervous activity; however, these markers are indirect indicators of autonomic nervous activity. Therefore, the activity evaluated based on heart rate variability and blood pressure variability may limit the reliability and validity of research results. The use of direct indicators of the activity such as muscle sympathetic nerve activity (Goso et al., 2001), plasma epinephrine and norepinephrine concentrations (Kasahara et al., 2006), and baroreflex sensitivity (Bernardi et al., 2002) is worth exploring as evaluation markers in future research in order to improve the reliability and validity of analytical results of autonomic nervous activity monitored during breathing in patterns tested in the present study.

In the preceding studies that investigated the differences in cardiopulmonary responses during exercise between upright and recumbent bicycle ergometers, rate pressure product and rating of perceived exertion were higher at low intensity, whereas blood pressure, $\dot{V}_E$, and $\dot{V}_E/\dot{V}_{CO_2}$ were higher from low to high intensity when a recumbent bicycle ergometer was used rather than an upright bicycle ergometer (Quinn et al., 1995; Saitoh et al., 2005). The rate pressure product and rating of perceived exertion might have been lower at low intensity, whereas blood pressure, $\dot{V}_E$ and $\dot{V}_E/\dot{V}_{CO_2}$ might have been lower from low to high intensity, if the present study was designed using an upright bicycle ergometer instead of a recumbent one.

In the present study, striking effects of prolonged expiration breathing included the suppression of increases in blood pressure associated with decreased sympathetic and increased parasympathetic nervous activities during incremental exercise. In addition, prolonged expiration breathing enhanced the efficiency of both ventilation and exercise, and it might have suppressed the exercise-induced increase in myocardial $\dot{V}_{O_2}$ in healthy young male volunteers.

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