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低強度・長時間水泳運動トレーニングによりラット骨格筋で発現するタンパク質のプロテオミクス;2D-DIGE解析

山口 航[1,2) 藤本恵理[1,2) 樋口 満[3) 田畑 泉[1,4)

A DIGE PROTEOMIC ANALYSIS FOR LOW-INTENSITY EXERCISE-TRAINED RAT SKELETAL MUSCLE

WATARU YAMAGUCHI, ERI FUJIMOTO, MITSURU HIGUCHI and IZUMI TABATA

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Abstract

Background: Exercise training induces various adaptations in skeletal muscles. However, the mechanisms remain unclear. **Purpose:** Therefore, we conducted 2D-DIGE proteomic analysis, which has not yet been used for elucidating adaptations of skeletal muscle after low-intensity exercise training (LIT). **Methods:** For five days, rats performed LIT, which consisted of two 3-h swimming exercise with45-m rest between the exercise bouts. 2D-DIGE analysis was conducted on epitrochlearis muscles excised eighteen hours after the final training exercise. **Results:** Proteomic profiling revealed that, out of 681 detected and matched spots, 22 proteins exhibited changed expression by LIT compared with sedentary rats. All proteins were identified by MALDI-TOF/MS. **Conclusion:** The proteomic 2D-DIGE analysis following LIT identified expressions of skeletal muscle proteins, includingATPsyn α, UQCRC1, dihydrolipoamide dehydrogenase, dihydrolipoamide acetyltransferase, that were not previously reported to change their expressions after exercise-training.

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Key words : swimming, epitrochlearis, proteomics, low-intensity exercise training

I. 緒　　言

身体運動・トレーニングにより，骨格筋では様々な適応が起こることが知られている．例えば，持久力の向上，筋力の向上，筋肥大，糖・脂質代謝機能の向上，タンパク質・アミノ酸代謝回転の亢進，抗ストレス機構の亢進などが挙げられる．これらの適応現象は，運動・筋収縮に関係する様々なシグナルを介して最終的に生理学的機能をもつ分子，主にタンパク質の発現を高めていることが考えられる．しかしながら，それぞれの分子メカニズムについては不明な点が多い．

最近，運動科学・体力科学の分野において，運動によって骨格筋で発現変動が認められる分子を「網羅的解析」によって探索するという試みがなされている．その中でプロテオミクス手法(Proteomics)が知られている．これは，タンパク質を2次元電気泳動(2-DE)にて分離し，質量分析(MS)とデータベースを用いて網羅的に解析する技術である．これまで，この手法を用いて，持久的運動トレーニング[1)

[1)独立行政法人国立健康・栄養研究所健康増進プログラム
〒162-8636 東京都新宿区戸山1-23-1
[2)早稲田大学スポーツ科学研究科
〒359-1192 埼玉県所沢市三ヶ島2-579-15
[3)早稲田大学スポーツ科学学術院
〒359-1192 埼玉県所沢市三ヶ島2-579-15
[4)立命館大学スポーツ健康科学部
〒525-8577 滋賀県草津市野路東1-1-1

Health Promotion and Exercise Program, National Institute of Health and Nutrition
1-23-1 Toyama, Shinjuku, Tokyo 162-8636, Japan
Graduate School of Sport Science, Waseda University
2-579-15 Mikajima, Tokorozawa City, Saitama Prefecture, 359-1165, Japan
Faculty of Sport Sciences, Waseda University
2-579-15 Mikajima, Tokorozawa City, Saitama Prefecture, 359-1165, Japan
Faculty of Health and Sport Science, Ritsumeikan University
1-1-1 Noji-Higashi, Kusatsu City, Shiga Prefecture, 525-8577, Japan

や，一過性の水泳運動などで発現変動する新しいタンパク質の発見も観察されている[2,3]．しかしながら，これらの報告では２次元電気泳動における解析方法が古典的であり，低発現のタンパク質の同定や発現差異の定量性に関して課題があった．

近年，２次元電気泳動の発展系として，蛍光標識ディファレンシャルゲル電気泳動法(2-dimensional difference gel electrophoresis ; 2D-DIGE)を用いた研究が頻出している．このシステムでは，タンパク質サンプルを電気泳動前に蛍光標識することにより，微量なタンパク質の検出とゲル間の泳動誤差が改善され，２次元電気泳動法の限界であった定量性，再現性が著しく向上した[4]．我々は最近，この2D-DIGEを用いて，高強度・短時間トレーニング(high-intensity intermittent swimming training: HIT)によりラット骨格筋で発現するプロテオミクス解析を初めて試みた結果，NDUFS1やparvalbuminなど，これまで運動トレーニングによる生理学的な発現変動の報告がなされていなかったタンパク質の同定に成功した[5]．一方で我々は，低強度・長時間水泳運動トレーニング(low-intensity prolonged swimming training: LIT)を行うことにより，骨格筋で糖取り込み機能と強い関係のあるタンパク質である糖輸送体GLUT4の発現や，ミトコンドリア新生のマーカーであるクエン酸合成酵素(CS)活性などが最大限に増加することを報告している[6,7]．しかしながら，これまで，ミトコンドリアの酸化系酵素の発現量増加を含む運動トレーニングに対する骨格筋の適応機序研究に用いられている低強度長時間トレーニングにおいて，2D-DIGEによるトレーニング活動筋のタンパク質の定量的差異解析はこれまでなかった．

そこで，本研究では2D-DIGEを用い，低強度・長時間トレーニングによって発現変動するタンパク質の網羅的解析を検討した．

Ⅱ．方　　法

A．飼育条件

実験動物として４週齢(体重70～80g)の雄性Sprague-Dawley系ラットを日本クレア社より購入した．ラットは室温22±2℃，湿度60±5℃に保たれた飼育室にて，実験動物用固形飼料(日本クレア社製：CE-2)および飲料水を自由摂取させながら飼育した．さらに，無負荷で３時間の水泳運動を45分の休息を挟んで２セット行う低強度・長時間水泳運動トレーニング(low-intensity prolonged swimming training: LIT)群と，非運動群として終日ケージに入れておくコントロール(CON)群の２群に５匹ずつ無作為に分けた．

本研究は独立行政法人国立健康・栄養研究所倫理委員会での審査を受け，実施承諾を得て行われた．

B．運動プロトコルと筋サンプル採取

トレーニング期間は連続した５日間とし，１日１回の頻度で行った．90L容量のポリバケツに水深45cmまで35±1℃の温水を入れ，LIT群のラットに対し無負荷で３時間の水泳運動を45分の休憩を挟んで２セット(計360分)行わせた．ラットは６匹同時に泳がせた．このようにすることにより，ほぼ同一の運動量を各々のラットに確保することが可能となる．本研究ではすべてのトレーニングを６匹同時に同一バケツで泳がせたが，他の研究で泳がせる匹数が６匹未満の場合は，ダミーのラットを一緒に同一バケツで泳がせ，常に泳いでいる匹数を６匹とするようにしている．２つの水槽を用意しておき，水温が34℃より低下した場合には，35℃のお湯をはった新しいバケツにラットを移し，水泳運動を継続させた．同様のプロトコルによる低強度・長時間トレーニングによりミトコンドリアの酸化系酵素及びGLUT4等のタンパク質の有意な発現量増加が見られることが報告されている[8,9]．

最終トレーニング終了の翌日(18時間後)に，両群のラットに体重100g当たり5mgのペントバルビタール・ナトリウムネンブタール注射液を腹腔投与し麻酔した後，前肢筋である滑車上筋(Epitrochlearis)を摘出した．滑車上筋は，速筋線維優位(TypeⅡ fiber: ~90%)であり，本研究で用いた水泳運動トレーニングによってこの骨格筋が動員されることが数々の研究で報告されている[7,10]．摘出した筋サンプルは液体窒素にて凍結させ，分析まで-80℃で保存した．

凍結された滑車上筋は，RIPA buffer [50mM Tris-HCl pH 7.4, 1 % Nonidet P-40, 0.25% sodium deoxycholate, 150mM NaCl, 1mM EDTA, 1mM NaF, 1mM sodium orthovanadate(Na_3VO_4), 2μl/ml Protease Inhibitor Cocktail]で氷中にてホモジ

ナイズし，遠心後の上清をサンプルとした．得られたサンプルのタンパク質量は2D-Quant kit(GE Healthcare社製)にて定量した．

C．蛍光ディファレンスゲル２次元電気泳動(2D-DIGE)と画像解析

2D-DIGEはGE Healthcare社のプロトコルに従って行った．タンパク質サンプルは蛍光色素(CyDye DIGE fluor minimal dye)であるCy3, Cy5により暗所で30分間，氷上で標識した(50 μg protein/400pmol dye)．また，内部標準としてすべてのサンプルを等量ずつ混合したものをCy2蛍光色素で標識した．標識したサンプルにバッファー[7M urea, 2M thiourea, 4% w/v CHAPS, 2.4% v/v DeStreak Reagent, 1% v/v IPG buffer pH 3-10]を添加した後，固定化pH勾配ゲルであるIPG Drystrip [24cm, pH 3-10, linear (GE Healthcare社)]に添加し，IPGphor 3 Isoelectric Focusing Unit (GE Healthcare社)を用いて等電点電気泳動を行った．次に，Ettan DALT*six* Large Electrophoresis System (GE Healthcare社)を用いて分子量による２次元目の泳動を行った．

電気泳動後，Typhoon 9400 scanner (GE Healthcare社)により，それぞれCy2, Cy3, Cy5の各最適励起波長で各ゲルを100 μmの解像度で取り込んだ．画像解析は，DeCyder V. 5.0 software (GE Healthcare社)を用いて，製造元の推奨プログラムに従って行った(Marouga et al. 2005; Karp et al. 2004)．Differential In-gel Analysis(DIA)およびBatch Processorモジュールによりスポット検出，定量解析を行い，最終的にBiological Variation Analysis(BVA)モジュールを用いて内部標準サンプルを介したゲル間のスポットマッチングおよび定量値の標準化，Student's *t*-testによる有意差検定を行った．

D．タンパク質のトリプシン消化

約1 mm^3の大きさに泳動ゲルのスポットを切り出し，Shevchenko et al.[11]の方法を一部修正してタンパク質のゲル内消化を行った． タンパク質の消化酵素として，トリプシン溶液[12.5ng/μL sequencing grade modified trypsin (Promega社), 50mM NH_4HCO_3, 5mM $CaCl_2$]をサンプルゲルに添加し，氷上でインキュベーションした．ゲル内で消化されたペプチドは20mM NH_4HCO_3, 5%ギ酸/50% ACN溶液を用いて回収し，その回収した抽出液をSpeed Vacで濃縮した．

E. 質量分析とPMF(peptide mass fingerprinting)法によるタンパク質の同定

得られたペプチド抽出液に対し，ZipTip μ-C18ピペットチップ(Millipore社)を用いて脱塩処理を行った．質量分析機はVoyager DE-STR(Applied Biosystems社)を用い，matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF/MS)解析を行った．タンパク質の同定には，Matrix Science社のMASCOT (http://www.matrixscience.com)を利用した．

F. バリデーション

ウエスタンブロット解析にてMS解析で同定されたタンパク質のバリデーションを行った．４週齢の雄性Sprague-Dawley系ラットにより，2D-DIGE解析と同様の運動トレーニングを行った群(n=6)とコントロール群(n=6)の滑車上筋のタンパク質を比較した．ホモジナイズされた滑車上筋をLaemmliサンプルバッファーに溶かし，SDS-PAGEにて電気泳動を行った後，PVDFメンブレンに転写した．転写したPVDFメンブレンは，一次抗体を５%スキムミルクで4℃一晩ブロッキングした後，２次抗体[HRP anti-rabbit；parvalbumin, F_1ATPase(ATPsynβ)]を室温60分反応させ，ECL plus(GE Healthcare社)にて化学発光させてバンドを検出した．

図表に示した値は平均値±標準偏差で表示した．２群の差の検定にはStudentの *t* 検定を用い，危険率５%未満を有意水準とした．

Ⅲ．結　　　果

低強度・長時間水泳運動トレーニング群(LIT)とコントロール群(CON)で，ラット滑車上筋において発現しているタンパク質を2D-DIGE法によってディファレンシャル解析を行った結果，すべてのゲルで約1000スポット検出され，その中で681スポットがすべてのゲル間でマッチして検出された．さらに，統計的に有意に1.3倍以上発現が増減したスポットが22個存在した(Fig. 1)．このうち，19スポットが発現増加し，残りの３スポットが発現減少していた．さらにMALDI-TOF/MSによって19個のタン

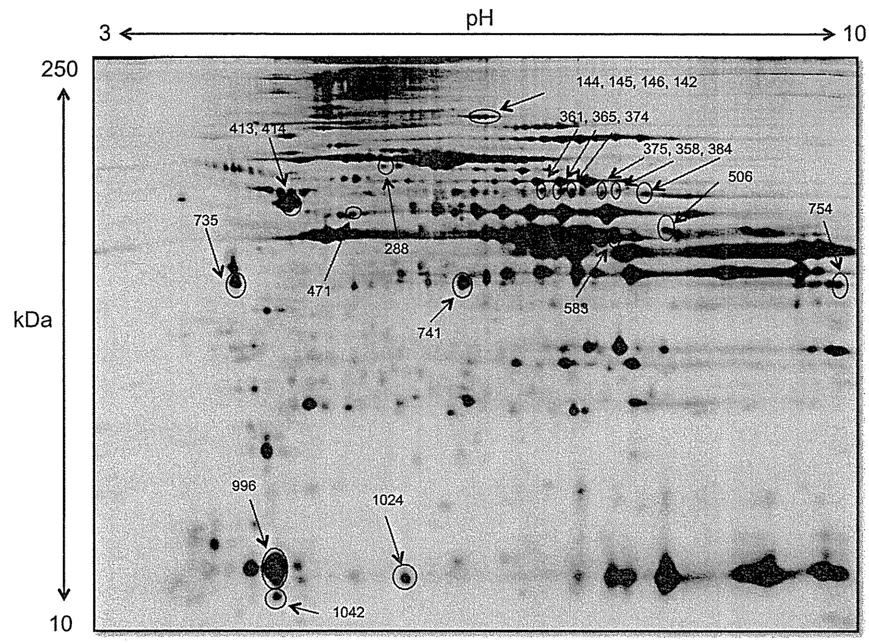

Figure 1. Epitrochlearis muscle protein profiling by 2D-DIGE. A typical 2D-pattern (Cy2-labeled) gel image of a 50-μg protein extract separated in a pH 3-10 IPG strip in the first dimension and 12.5 % polyacrylamide gel in the second. Automated image analysis by Decyder detected and matched 681 protein spots in single-gel images. Differential analysis of the epitrochlearis muscle extracts after low-intensity prolonged training (LIT) and control (CON) extracts, revealed 22 differentially expressed ($p < 0.05$) spots. Identified protein spots were labeled with numbers as they appear in the MS list (see Table 1.)

パク質の同定に成功した(Table 1.).

同定したタンパク質のうち，代謝系タンパク質16個の発現が増加した．それらは，ミトコンドリア電子伝達系酵素[ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit (ATP synthase α subunit; spots 374 and 358)，ATP synthase beta subunit(ATP synthase β subunit; spots 414 and 413)，ubiquinol-cytochrome c reductase core protein 1 (UQCRC1; spot 471)]，ミトコンドリア酸化系酵素[oxoglutarate (alpha-ketoglutarate) dehydrogenase (lipoamide) (OGDH; spots 142, 146, 145, and 144)，dihydrolipoamide dehydrogenase (spots 361 and 365)，dihydrolipoamide acetyltransferase (spot 288)]，脂肪酸結合タンパク質[fatty acid binding protein 3, muscle and heart (spot 1024)]，リンゴ酸-アスパラギン酸シャトル[Cytosolic aspartate aminotransferase (spot 583)，malate dehydrogenase, mitochondrial (spot 754)，malate dehydrogenase 1, NAD (soluble) (spot 741)]であった．

一方，収縮系タンパク質[striated-muscle alpha tropomyosin (spot 735)]や，その他[parvalbumin (spot 996)，Chain 1, Refined X-Ray Structure Of Rat Parvalubumin (spot 1042)]のタンパク質の発現が減少した．

2D-DIGE解析の結果を確認するため，LIT後に有意に発現変動したタンパク質をウエスタンブロット解析した(Fig. 2)．その結果，ATP synthase β subunit，parvalbuminもウエスタンブロット解析により2D-DIGE解析と同様な結果が確認された．

Ⅳ. 考　　察

本研究の2D-DIGE解析により，低強度・長時間運動トレーニング(LIT)後に変化する多くの骨格筋タンパク質が初めて明らかとなった．ラット滑車上筋において発現しているタンパク質のうち，2D-DIGEによって681スポットがすべてのゲル間でマッチして検出された．これは，我々が行った先行研究のスポット検出数；800スポットと同レベルである[5]．すなわち，通常の染色方法(CBBまたは銀染色)による標準的な２次元電気泳動を用いた他の研究よりもはるかに検出数が大きい[12]．Burniston[1]のプロテオーム研究では，ラットにおいて中強度

Table 1. List of DIGE-identified proteins with a changed expression level following low-intensity prolonged training (LIT) in rat epitrochlearis muscle

Protein function (Classification)	Master No. (LIT)	gi No.	Protein name	Molecular mass (kDa)	Isoelectric point (pI)	Sequence coverage (%)	Average ratio V_E/V_C ※ (Fold change)	t-Test *p*-value
Metabolic prtoeins	374	149029483	ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit, isoform 1, isoform CRA_d	54.6	8.2	27	1.79	1.80E-04
	358	149029483	ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit, isoform 1, isoform CRA_d	54.6	8.2	32	1.73	8.40E-04
	414	1374715	ATP synthase beta subunit	51.2	4.9	25	1.33	6.60E-03
	413	1374715	ATP synthase beta subunit	51.2	4.9	25	1.31	1.80E-02
	471	51948476	ubiquinol-cytochrome c reductase core protein 1	53.5	5.6	20	1.30	8.70E-03
	142	62945278	oxoglutarate (alpha-ketoglutarate) dehydrogenase (lipoamide)	117	6.3	17	1.53	6.50E-04
	146	62945278	oxoglutarate (alpha-ketoglutarate) dehydrogenase (lipoamide)	117	6.3	22	1.43	2.30E-04
	145	62945278	oxoglutarate (alpha-ketoglutarate) dehydrogenase (lipoamide)	116	6.3	12	1.42	1.50E-03
	144	62945278	oxoglutarate (alpha-ketoglutarate) dehydrogenase (lipoamide)	117	6.3	22	1.37	4.00E-03
	361	40786469	dihydrolipoamide dehydrogenase	54.6	8.0	24	1.46	3.50E-04
	365	40786469	dihydrolipoamide dehydrogenase	54.6	8.0	21	1.38	1.00E-03
	288	220838	dihydrolipoamide acetyltransferase	57.6	5.5	11	1.31	7.50E-03
	1024	13162363	fatty acid binding protein 3, muscle and heart	14.8	5.9	33	1.55	1.40E-02
	583	220684	Cytosolic aspartate aminotransferase	46.6	6.7	36	1.39	7.10E-04
	754	42476181	malate dehydrogenase, mitochondrial	36.1	8.9	46	1.30	4.70E-03
	741	15100179	malate dehydrogenase 1, NAD (soluble)	36.6	6.2	31	1.30	1.70E-03
Contractile protein	735	207349	striated-muscle alpha tropomyosin	32.7	4.7	40	-1.36	9.40E-03
Other	996	11968064	parvalbumin	11.9	5.0	79	-1.32	5.20E-03
	1042	494573	Chain 1, Refined X-Ray Structure Of Rat Parvalubumin, A Mammalian Alpha-Lineage Parvalbumin, At 2.0 A Resolution	11.8	5.0	53	-1.32	2.00E-02

※ V_E/V_C indicates the value ratio derived from the normalized spot volume standardized against the intra-gel standard provided by DeCyder software analysis.

(70~75% $\dot{V}O_2$peak), 30分/日, 4日/週, 7週間の走行運動トレーニング後, 足底筋(plantaris; 速筋線維優位)で発現変動したミトコンドリアタンパク質は, TCA回路の酵素であるaconitase 2, succinate CoAのみ(2 spots)であった. 本研究との結果の違いは, Burniston[1]の研究では感度の低いCBB染色及び通常の2次元電気泳動法を使用していることが原因と考えられる. また, 運動条件に関しては, 強度を除けば本研究のほうが先行研究より総運動時間が2倍も長いことも原因の一つとして考えられるかもしれない.

ミトコンドリア電子伝達系酵素であるATP synthase α subunit, ATP synthase β subunit, UQCRC1, ミトコンドリア酸化系酵素であるOGDH, dihydrolipoamide dehydrogenaseとdihydrolipoamide acetyltransferase, 計6種類のタンパク質が, CON群に比べ有意に約1.3~1.8倍発現増加した($p<0.05$). このうち, ATP synthase β subunit, OGDHについてはこれまでに運動トレーニングで増加する報告がある[13,14]. ATP synthase β subunitは本研究でのウエスタンブロット解析でもCON群と比べLIT群で有意に発現増加することを確認した(Fig. 2A).

ATP synthase α subunit, UQCRC1, dihydrolipoamide dehydrogenase, dihydrolipoamide acetyltransferaseは初めて運動トレーニングで増加することが明らかとなった. ミトコンドリア内膜では, 呼吸鎖を構成する複合体(Ⅰ～Ⅳ)を介して電子を伝達し, それに伴い生じたプロトン(H^+)濃度勾配を利用して複合体ⅤによりADPからATPを合成する. UQCRC1は, UQCRC2とともに電子をシトクロムcに渡す機能をもつ電子伝達系酵素複合体Ⅲを構成するサブユニットであり, UQCRC2は運動トレーニングで発現増加することが報告されている[15]. ATP synthase α subunitは, ATP synthase β subunitと同じくADPと結合してATPを合成する機能をもつ電子伝達系酵素複合体Ⅴの構成するサブユニットである. すなわち, LITによってミトコンドリア電子伝達系酵素複合体Ⅲ, Ⅴを構成するタンパク質がそれぞれ発現増加し, この二つの電子伝達系酵素複合体の発現が増加することが確認された.

Fatty acid binding protein 3, muscle and heart(FABP3)は, LIT群がCON群に比べ有意に1.55

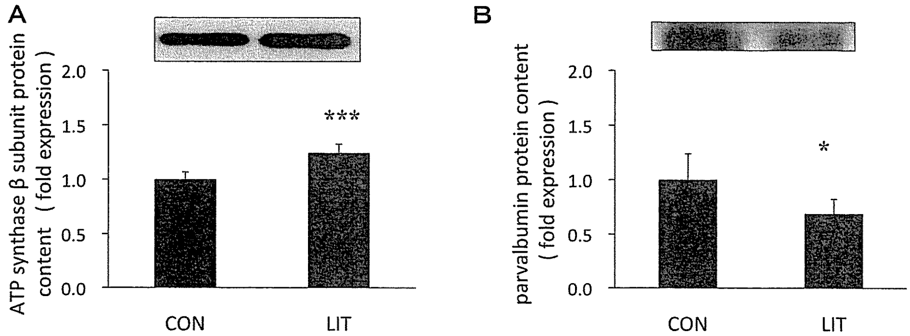

Figure 2. Western blot analysis of selected muscle proteins following low-intensity prolonged training (LIT). Identical immunoblots labeled with antibodies to F_1-ATPase (; ATPsyn β) (A) and parvalbumin (B). '*' and '***' indicate significant differences from results in the control group (CON) at levels of $p < 0.05$ and $p < 0.001$. Values are means ± SD for six muscles.

倍発現増加した (p<0.05). このタンパク質は，既に運動・トレーニングによって発現増加することが知られている[16]. すなわち，LITによって骨格筋FABP3の発現量が増加し，遊離脂肪酸の取り込みが増加する可能性が考えられる.

Cytosolic aspartate aminotransferaseと，malate dehydrogenase 1, NAD, malate dehydrogenase, mitochondrialが，LIT群でCON群に比べ有意に約1.3~1.4倍発現増加した (p<0.01). 運動トレーニングに関して，これらの酵素が増加することは過去に報告されている[17]. これらの酵素は，ミトコンドリア外のNADHのエネルギー (電子)を，電子伝達系で利用するためにミトコンドリア内へ転送する役割を担うリンゴ酸－アスパラギン酸シャトル(MA shuttle)に関するタンパク質である. すなわち，LITにより筋中のMA shuttleに関わる酵素が発現増加したことが明らかとなった.

収縮系タンパク質であるstriated-muscle alpha tropomyosinが，LIT群でCON群に比べ有意に1.36倍発現減少した(p<0.01). Tropomyosin(トロポミオシン)はアクチン上に存在するタンパク質であり，トロポニンに結合し,筋収縮の制御に関与している. 骨格筋では，本研究で同定されたαトロポミオシンとβトロポミオシンの２種類のアイソフォームがあり，遅筋線維に比べて速筋線維ではαタイプの発現量が高く，α/βの比が高いことも知られている[18]. すなわち，LITによってαタイプのトロポミオシンの発現量が減少したのは，速筋線維優位である滑車上筋での筋線維組成の変化によるのかもしれない.

本研究により，LIT群でparvalbuminがCON群に比べ有意に1.32倍発現が減少した(p<0.01). さらに，この結果はウエスタンブロット解析でも確認された(Fig. 2B). parvalbuminは，細胞質に局在する高親水性カルシウム結合タンパク質であり，骨格筋においては遅筋線維と比べ速筋線維で高濃度に存在する[19]. したがって，本研究の結果は，低強度の運動トレーニングによる筋線維組成の変化によるものと考えられる.

実際に骨格筋に発現しているタンパク質は50000以上と推測されるが，本研究で発現が確認されたのは1000スポット程度であり，コントロール群とトレーニング群でマッチングが確認されたのは681個のタンパク質である. したがって，本プロテオミクス法はすべてのタンパク質を網羅的に解析できていない. 多くのトレーニング実験で報告されているGLUT4やクエン酸合成酵素のようなタンパク質は，本研究では発現及びその変化も確認することはできなかった. これは二次元電気泳動を用いたプロテオミクス法の限界であると考えられ，多くのタンパク質が二次元電気泳動ゲルから散逸していると推測される.

プロテオミクス法を用いて，長時間水泳トレーニングにより発現量が変化した骨格筋タンパク質について，抗体が市販され，抗体が入手可能なタンパク質について，ウエスタンブロット法を用いて，プロテオミクス法のバリデーションを行った. 今後は，

抗体が手に入り次第，ウエスタンブロット法によるバリデーションを行う必要がある．

本研究では低強度長時間運動トレーニングとして水泳運動を採用した．水泳運動は長時間水中に浸水することより，運動の影響と水浸の影響が加わっている可能性がある．しかし，同様な水泳運動トレーニングと同時間・同温度の水浸がミトコンドリアタンパク質の新生に影響を与えると推測されるperoxisome proliferator-activated receptor γ coactivator-1 α（PGC-1α）のmRNAの発現量に水浸が影響を与えてないことが報告されている[20]．また，同様の水浸プロトコルによりGLUT4及びミトコンドリアのタンパク質の発現変化がないことも確認しており（未発表），少なくともこのような場合の骨格筋タンパク質発現の変化は，水浸ではなく，水泳トレーニングによる変化であると考えられる．今後，水浸による骨格筋タンパク質発現の変化を観察する実験研究を実施して，水浸の影響をみる必要もあると考えられる．

また長時間の水泳運動は，いわゆる"ストレス"を実験動物に与え，その影響が骨格筋のプロテオームに影響を与える可能性がある．しかし，同様な長時間水泳トレーニングにより水泳運動により動員されない骨格筋（ラット後肢筋であるヒラメ筋）では，動員される筋である滑車上筋で発現が見られるGLUT4やPGC-1αの発現が認められていないことなどから[9,21]，"ストレス"による内分泌系の変動による影響は限定的であると推測される．

V．まとめ

本研究により，低強度・長時間運水泳動トレーニング後の活動筋のタンパク質発現量の変化をプロテオミクス法により網羅的に分析したところ，従来，先行研究により報告のないミトコンドリア電子伝達系酵素であるATP synthase α subunit, UQCRC1及び，ミトコンドリア酸化系酵素であるdihydrolipoamide dehydrogenase, dihydrolipoamide acetyltransferaseを含む22個のタンパク質の発現量の変化が認められた．

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REGULAR ARTICLE

Evaluation of anthropometric parameters and physical fitness in elderly Japanese

Nobuyuki Miyatake · Motohiko Miyachi · Izumi Tabata · Takeyuki Numata

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Abstract

Objectives We evaluated anthropometric parameters and physical fitness in elderly Japanese.

Methods A total of 2,106 elderly Japanese (749 men and 1,357 women), aged 60–79 years, were enrolled in a cross-sectional investigation study. Anthropometric parameters and physical fitness, i.e., muscle strength and flexibility, were measured. Of the 2,106 subjects, 569 subjects (302 men and 267 women) were further evaluated for aerobic exercise level, using the ventilatory threshold (VT).

Results Muscle strength in subjects in their 70s was significantly lower than that in subjects in their 60s in both sexes. Two hundred and twenty-nine men (30.6%) and 540 women (39.8%) were taking no medications. In men, anthropometric parameters were significantly lower and muscle strength, flexibility, and work rate at VT were significantly higher in subjects without medications than these values in subjects with medications. In women, body weight, body mass index (BMI), and abdominal circumference were significantly lower, and muscle strength was significantly higher in subjects without medications than these values in subjects with medications.

Conclusion This mean value may provide a useful database for evaluating anthropometric parameters and physical fitness in elderly Japanese subjects.

Keywords Elderly Japanese · Anthropometric parameters · Muscle strength · Ventilatory threshold (VT)

Introduction

The proportion of elderly people (over the age of 65 years) in Japan has increased and this has become a public health challenge in Japan. For example, in Japan, 28,216,000 people (22.1% of the population) are reported to be over the age of 65 [1].

It has been shown that obese subjects have a high mortality rate [2] and have associated atherogenic risk factors, such as hypertension, coronary heart disease, diabetes mellitus, and dyslipidemia [3, 4]. In addition, Sandvik et al. reported that physical fitness was a graded, independent, long-term predictor of mortality from cardiovascular causes in healthy, middle-aged men [5]. Also, Metter et al. [6] reported that lower and declining muscle strength was associated with increased mortality, independent of physical activity and muscle mass. In order to provide proper management and control of anthropometric parameters and physical fitness in elderly Japanese, precise assessments of these parameters are necessary. However, the evaluation of anthropometric parameters and physical fitness still remains to be investigated in elderly Japanese who are not taking medications.

N. Miyatake (✉)
Department of Hygiene, Faculty of Medicine,
Kagawa University, Miki, Kagawa 761-0793, Japan
e-mail: miyarin@med.kagawa-u.ac.jp

M. Miyachi
National Institute of Health and Nutrition,
Tokyo 162-8636, Japan

I. Tabata
Sports and Health Science, Ritsumeikan University,
Kusatsu, Shiga 525-8577, Japan

T. Numata
Okayama Southern Institute of Health,
Okayama Health Foundation, Okayama 700-0952, Japan

Therefore, we evaluated anthropometric parameters and physical fitness in elderly Japanese and compared these parameters in subjects with and without medications.

Subjects and methods

Subjects

We used data for all 2,106 elderly subjects (749 men and 1,357 women), aged 60–79 years, among 16,383 subjects in a cross-sectional investigation study. All subjects met the following criteria: (1) they had been wanting to change their lifestyle i.e., diet and exercise habits, and had received an annual health checkup between June 1997 and December 2009 at Okayama Southern Institute of Health; (2) their anthropometric, muscle strength, and flexibility measurements had been taken as part of their annual health checkups; and (3) they provided written informed consent (Table 1).

In a second analysis, among the 2,106 subjects, we further examined the data on 569 subjects (302 men and 267 women) who undertook measurements of aerobic exercise level; we also examined anthropometric, muscle strength, and flexibility measurements in these second-analysis subjects (Table 2).

The study was approved by the Ethics Committee of Okayama Health Foundation.

Athropometric measurements

The anthropometric parameters were evaluated by using the following parameters: height, body weight, body mass index (BMI), abdominal circumference, and hip circumference. BMI was calculated as weight/[height]^2 (kg/m^2). The abdominal circumference was measured at the umbilical level and the hip was measured at the widest circumference over the trochanter in standing subjects after normal expiration [7].

Muscle strength

To assess muscle strength, grip and leg strength were measured. Grip strength was measured using the THP-10 (SAKAI, Tokyo, Japan) device, while leg strength was measured with a dynamometer (COMBIT CB-1; MINATO Co., Osaka, Japan). Isometric leg strength was measured as follows: the subject sat in a chair, grasping the armrest in order to fix the body position. The dynamometer was then attached to the subject's ankle joint with a strap. Next, the subject extended the leg to 60° [8]. To standardize the influence of the total body weight, we calculated the muscle strength (kg) per body weight (kg) [9].

Flexibility

Flexibility was measured as follows in all the participants. Sit-and-reach measurements were obtained to assess the overall flexibility in forward flexion, with the measurements recorded as the distance (in cm) between the fingertips and toes. The subject's knees were kept straight throughout the test and ankles were maintained at 90° by having the soles of the feet pressed against a board perpendicular to the sitting surface [10].

Oxygen uptake at ventilatory threshold (VT)

A graded ergometer exercise protocol [11] had been carried out at the subjects' checkups. After breakfast (2 h), resting

Table 1 Clinical profiles of subjects enrolled in the first analysis

	Men (n = 749)			Women (n = 1,357)		
	Mean ± SD	Minimum	Maximum	Mean ± SD	Minimum	Maximum
Age (years)	65.6 ± 4.6	60	79	64.9 ± 4.2	60	79
Height (cm)	164.4 ± 5.5	145.3	180.2	151.9 ± 5.0	136.2	167.0
Body weight (kg)	65.9 ± 9.3	40.1	112.2	55.3 ± 7.9	33.4	97.3
Body mass index (kg/m^2)	24.3 ± 3.0	16.2	40.9	24.0 ± 3.2	15.4	41.9
Abdominal circumference (cm)	86.1 ± 9.2	61.6	127.0	78.8 ± 9.3	54.7	121.6
Hip circumference (cm)	91.9 ± 5.5	77.8	122.7	90.3 ± 5.4	69.0	120.5
Right grip strength (kg)	36.4 ± 7.0	8.7	60.0	22.3 ± 4.6	4.9	39.9
Left grip strength (kg)	35.0 ± 6.9	5.0	55.7	21.4 ± 4.5	4.3	47.4
Leg strength (kg)	51.0 ± 13.4	11.7	97.0	35.3 ± 8.6	10.7	69.7
Leg strength per body weight	0.78 ± 0.19	0.20	1.50	0.65 ± 0.17	0.15	1.26
Flexibility (cm)	0.6 ± 10.3	−34.0	28.3	11.2 ± 8.1	−22.0	28.4
Number of subjects without medications (%)	229 (30.6)			540 (39.8)		

Table 2 Clinical profiles of subjects enrolled in the second analysis

	Men ($n = 302$)			Women ($n = 267$)		
	Mean ± SD	Minimum	Maximum	Mean ± SD	Minimum	Maximum
Age (years)	65.3 ± 4.3	60	79	64.7 ± 4.0	60	77
Height (cm)	164.6 ± 5.1	149.1	178.3	152.4 ± 4.2	142.2	164.2
Body weight (kg)	68.6 ± 9.7	47.6	112.2	59.3 ± 8.8	37.6	97.3
Body mass index (kg/m^2)	25.3 ± 3.1	18.9	40.9	25.5 ± 3.7	16.7	41.9
Abdominal circumference (cm)	88.8 ± 9.8	62.5	127.0	83.4 ± 10.6	60.0	121.6
Hip circumference (cm)	93.2 ± 6.0	79.7	122.7	92.4 ± 6.4	72.5	120.5
Right grip strength (kg)	36.3 ± 6.8	13.4	60.0	22.1 ± 4.7	6.6	34.9
Left grip strength (kg)	35.1 ± 6.4	15.6	54.1	21.2 ± 4.5	6.9	33.0
Leg strength (kg)	51.0 ± 13.1	19.0	92.0	35.2 ± 9.0	11.0	69.7
Leg strength per body weight	0.75 ± 0.19	0.26	1.17	0.60 ± 0.15	0.16	1.08
Flexibility (cm)	−0.9 ± 10.1	−34.0	23.7	9.7 ± 8.3	−22.0	26.4
Oxygen uptake at VT (ml/kg/min)	12.5 ± 2.0	5.9	21.6	11.9 ± 1.7	7.6	16.8
Work rate at VT (watt)	53.6 ± 13.8	5.0	100.0	38.7 ± 10.5	5.0	70.0
Heart rate at VT (beats/min)	95.7 ± 12.9	64.0	146.0	99.0 ± 12.8	67.0	137.0
Number of subjects without medications (%)	33 (10.9)			55 (20.6)		

VT ventilatory threshold

ECG was recorded and blood pressure was measured. All subjects were then given a graded exercise after 3 min of pedaling on an unloaded bicycle ergometer (Excalibur V2.0; Lode, Groningen, The Netherlands). The profile of incremental workloads was automatically defined by the methods of Jones et al. [11], in which the workloads reach the predicted maximum rate of oxygen consumption ($\dot{V}O_{2max}$) in 10 min. A pedaling cycle of 60 rpm was maintained. Loading was terminated when the appearance of symptoms forced the subject to stop. During the test, ECG was monitored continuously, together with recording of the heart rate. Expired gas was collected, and rates of oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were measured breath-by-breath using a cardiopulmonary gas exchange system (Oxycon Alpha; Mijnhrdt, The Netherlands). The VT was determined by the standards of Wasserman et al. [12] and Davis et al. [13], and the V-slope method of Beaver et al. [14] from $\dot{V}O_2$, $\dot{V}CO_2$, and minute ventilation (VE).

Medications

The data on medications were obtained at interviews conducted by well-trained staff using a structured method. The subjects were asked if they were currently taking medications, i.e., those for diabetes, hypertension, dyslipidemia, and/or orthopedic diseases. When the answer was "yes", they were classified as subjects with medications. When the answer was "no", they were classified as subjects without medications.

Statistical analysis

Data are expressed as means ± standard deviation (SD) values. There were sufficient numbers of subjects, except for subjects in their 70s without medications in the second analysis. A comparison of parameters between subjects in their 60s and those in their 70s, between subjects with and without medications, and between subjects in their 60s and those in their 70s without medications was made using an unpaired t-test: $p < 0.05$ was considered to be statistically significant.

Results

Clinical profiles of the subjects in the first and second analyses are summarized in Tables 1 and 2. Two hundred and twenty-nine men (30.6%) and 540 women (39.8%) in the first analysis and 33 men (10.9%) and 55 women (20.6%) in the second analysis were not taking medications.

We compared the clinical parameters between subjects in their 60s and those in their 70s (Table 3). In men, height, body weight, BMI, and hip circumference in those in their 60s were significantly higher than the values in men in their 70s. However, abdominal circumference in men in their 60s was similar to that in men in their 70s. Muscle strength, flexibility, oxygen uptake at VT, work rate at VT, and heart rate at VT in men in their 60s were higher than the values in men in their 70s. In women, height was significantly

Table 3 Changes in anthropometric and physical fitness parameters in the first and second analyses in all subjects

	Men			Women		
	60–69	70–79	*p*	60–69	70–79	*p*
First analysis						
Number of subjects	604	145		1,158	199	
Height (cm)	164.8 ± 5.3	162.7 ± 5.9	**<0.0001**	152.2 ± 4.9	149.9 ± 4.9	**<0.0001**
Body weight (kg)	66.5 ± 9.0	63.3 ± 9.9	**0.0002**	55.3 ± 7.8	54.8 ± 7.9	0.3793
Body mass index (kg/m^2)	24.5 ± 3.0	23.9 ± 3.0	**0.0313**	23.9 ± 3.2	24.4 ± 3.3	**0.0373**
Abdominal circumference (cm)	86.2 ± 9.1	85.6 ± 9.5	0.4998	78.3 ± 9.1	81.9 ± 10.2	**<0.0001**
Hip circumference (cm)	92.2 ± 5.4	90.7 ± 5.6	**0.0030**	90.3 ± 5.4	90.0 ± 5.4	0.5006
Right grip strength (kg)	37.3 ± 6.8	32.6 ± 6.9	**<0.0001**	22.5 ± 4.5	20.9 ± 4.4	**<0.0001**
Left grip strength (kg)	36.0 ± 6.6	30.8 ± 6.5	**<0.0001**	21.6 ± 4.4	20.0 ± 4.3	**<0.0001**
Leg strength (kg)	53.2 ± 12.9	41.9 ± 11.3	**<0.0001**	35.9 ± 8.5	31.8 ± 8.7	**<0.0001**
Leg strength per body weight	0.81 ± 0.19	0.67 ± 0.19	**<0.0001**	0.66 ± 0.16	0.59 ± 0.17	**<0.0001**
Flexibility (cm)	1.0 ± 9.9	−1.1 ± 11.3	**0.0278**	11.4 ± 8.0	10.7 ± 8.4	0.3205
Second analysis						
Number of subjects	255	47		228	39	
Oxygen uptake at VT (ml/kg/min)	12.7 ± 1.9	11.5 ± 1.7	**<0.0001**	12.0 ± 1.7	11.1 ± 1.5	**0.0024**
Work rate at VT (watt)	56.0 ± 12.6	40.4 ± 12.5	**<0.0001**	40.2 ± 10.2	29.7 ± 8.8	**<0.0001**
Heart rate at VT (beats/min)	96.6 ± 12.5	91.2 ± 14.0	**0.0080**	99.3 ± 12.8	96.9 ± 12.7	0.2747

Values are means ± SD. *p* values in boldface are significant

VT ventilatory threshold

greater, and BMI and abdominal circumference were significantly lower in those in their 60s than the values in women in their 70s. Muscle strength, oxygen uptake at VT, and work rate at VT in those in their 60s were significantly higher than the values in women in their 70s.

We further analyzed clinical parameters, comparing them between subjects with and without medications (Table 4). There were significant differences in anthropometric parameters (except for height), muscle strength, flexibility, and work rate at VT between men with and without medications. In women, there were also significant differences in body weight, BMI, abdominal circumference, and muscle strength between the two groups.

In addition, in men in their 60s, muscle strength and flexibility in subjects without medications were significantly higher than these values in subjects with medications. In women, body weight, BMI, abdominal circumference, and hip circumference were significantly lower, and grip strength and leg strength per body weight were significantly higher in subjects without medications than these values in subjects with medications (Table 4).

In men in their 70s, anthropometric parameters were significantly lower and leg strength per body weight was significantly higher in men without medications than these values in men with medications. Muscle strength in women without medications was significantly higher than that in women with medications (Table 4).

We found that there were significant differences in some parameters between subjects with and without medications. We finally compared parameters between subjects in their 60s and subjects in their 70s in without medications (Table 5). In men, anthropometric parameters and muscle strength in those in their 70s were significantly lower than these values in men in their 60s. In women, only abdominal circumference in those in their 70s was higher than that in women in their 60s. There were no differences in other parameters between subjects in their 60s and those in their 70s.

Discussion

We evaluated anthropometric parameters, muscle strength, flexibility, and aerobic exercise levels in elderly Japanese. Especially in elderly subjects without medications, this mean value for those in their 60s and 70s may provide a useful database for evaluating anthropometric parameters and physical fitness.

It has been well reported that there is significant loss in muscle strength with aging [15, 16]. Aging is associated with alterations in body composition; there is an increase in body fat percentage and a concomitant decline in lean body mass [17]. Aging, therefore, results in substantial alterations in body composition, with a marked reduction in

Table 4 Comparison of anthropometric and physical fitness parameters between subjects with and without medications as classified by age groups

	Men			Women		
	Medication (−)	Medication (+)	*p*	Medication (−)	Medication (+)	*p*
All subjects						
First analysis						
Number of subjects	229	520		540	817	
Height (cm)	164.2 ± 5.4	164.5 ± 5.5	0.4997	152.0 ± 5.2	151.8 ± 4.9	0.3762
Body weight (kg)	64.6 ± 8.7	66.5 ± 9.5	**0.0097**	54.4 ± 7.6	55.8 ± 8.0	**0.0015**
Body mass index (kg/m^2)	23.9 ± 2.7	24.5 ± 3.1	**0.0076**	23.6 ± 3.1	24.2 ± 3.3	**0.0002**
Abdominal circumference (cm)	84.7 ± 9.0	86.7 ± 9.2	**0.0055**	77.4 ± 9.0	79.8 ± 9.4	**<0.0001**
Hip circumference (cm)	91.3 ± 5.0	92.2 ± 5.6	**0.0305**	89.9 ± 5.2	90.5 ± 5.5	0.0646
Right grip strength (kg)	37.5 ± 6.6	35.9 ± 7.2	**0.0028**	23.0 ± 4.4	21.8 ± 4.6	**<0.0001**
Left grip strength (kg)	35.8 ± 6.7	34.6 ± 7.0	**0.0213**	22.0 ± 4.2	21.0 ± 4.6	**<0.0001**
Leg strength (kg)	53.1 ± 12.8	50.2 ± 13.5	**0.0059**	36.2 ± 8.0	34.7 ± 9.0	**0.0013**
Leg strength per body weight	0.83 ± 0.19	0.76 ± 0.19	**<0.0001**	0.67 ± 0.15	0.63 ± 0.17	**<0.0001**
Flexibility (cm)	2.3 ± 10.8	−0.2 ± 9.9	**0.0023**	11.7 ± 8.0	11.0 ± 8.1	0.1189
Second analysis						
Number of subjects	33	269		55	212	
Oxygen uptake at VT (ml/kg/min)	12.9 ± 1.8	12.4 ± 2.0	0.2280	11.8 ± 1.6	11.9 ± 1.7	0.7549
Work rate at VT (watt)	59.0 ± 12.8	52.9 ± 13.8	**0.0176**	40.4 ± 9.2	38.2 ± 10.8	0.1651
Heart rate at VT (beats/min)	96.7 ± 9.9	95.6 ± 13.2	0.6505	99.7 ± 12.5	98.8 ± 12.8	0.6563
60–69						
First analysis						
Number of subjects	195	409		490	668	
Height (cm)	164.8 ± 5.0	164.8 ± 5.5	0.9816	152.2 ± 5.1	152.2 ± 4.8	0.8692
Body weight (kg)	65.7 ± 8.2	66.9 ± 9.4	0.1204	54.5 ± 7.6	56.0 ± 7.9	**0.0016**
Body mass index (kg/m^2)	24.2 ± 2.6	24.6 ± 3.1	0.0755	23.5 ± 3.1	24.1 ± 3.3	**0.0010**
Abdominal circumference (cm)	85.4 ± 8.8	86.6 ± 9.2	0.1416	77.2 ± 8.8	79.2 ± 9.2	**0.0002**
Hip circumference (cm)	91.8 ± 4.7	92.4 ± 5.7	0.1956	89.9 ± 5.2	90.6 ± 5.5	**0.0304**
Right grip strength (kg)	38.2 ± 6.2	36.8 ± 7.0	**0.0168**	23.1 ± 4.5	22.1 ± 4.5	**0.0006**
Left grip strength (kg)	36.8 ± 6.3	35.6 ± 6.7	**0.0427**	22.0 ± 4.3	21.3 ± 4.5	**0.0117**
Leg strength (kg)	54.7 ± 12.4	52.5 ± 13.1	**0.0472**	36.3 ± 8.0	35.5 ± 8.9	0.1073
Leg strength per body weight	0.84 ± 0.19	0.79 ± 0.19	**0.0028**	0.67 ± 0.15	0.64 ± 0.17	**0.0018**
Flexibility (cm)	2.5 ± 10.5	0.2 ± 9.6	**0.0087**	11.6 ± 8.0	11.1 ± 8.0	0.3002
Second analysis						
Number of subjects	30	225		53	175	
Oxygen uptake at VT (ml/kg/min)	12.9 ± 1.8	12.7 ± 1.9	0.5373	11.8 ± 1.7	12.1 ± 1.7	0.3367
Work rate at VT (watt)	59.5 ± 12.5	55.6 ± 12.6	0.1052	40.5 ± 9.4	40.1 ± 10.2	0.7872
Heart rate at VT (beats/min)	97.1 ± 10.2	96.5 ± 12.8	0.8199	99.6 ± 12.6	99.3 ± 12.8	0.8459
70–79						
First analysis						
Number of subjects	34	111		50	149	
Height (cm)	160.7 ± 6.3	163.3 ± 5.7	0.0243	150.4 ± 5.5	149.7 ± 4.7	0.4049
Body weight (kg)	58.2 ± 8.8	64.9 ± 9.7	**0.0005**	53.8 ± 7.4	55.1 ± 8.1	0.3041
Body mass index (kg/m^2)	22.5 ± 3.0	24.3 ± 2.9	**0.0025**	23.9 ± 3.5	24.6 ± 3.2	0.1770
Abdominal circumference (cm)	80.5 ± 9.4	87.2 ± 9.0	**0.0003**	80.0 ± 10.9	82.6 ± 9.9	0.1158
Hip circumference (cm)	88.2 ± 5.9	91.5 ± 5.3	**0.0029**	90.2 ± 4.7	90.0 ± 5.6	0.7944
Right grip strength (kg)	33.4 ± 7.4	32.3 ± 6.8	0.3916	22.7 ± 4.0	20.3 ± 4.4	**0.0006**
Left grip strength (kg)	30.6 ± 6.5	30.9 ± 6.6	0.8323	21.9 ± 3.9	19.4 ± 4.3	**0.0004**

Table 4 continued

	Men			Women		
	Medication (−)	Medication (+)	*p*	Medication (−)	Medication (+)	*p*
Leg strength (kg)	43.5 ± 11.3	41.5 ± 11.4	0.3593	34.8 ± 8.1	30.8 ± 8.6	**0.0043**
Leg strength per body weight	0.76 ± 0.21	0.65 ± 0.17	**0.0015**	0.65 ± 0.16	0.57 ± 0.16	**0.0010**
Flexibility (cm)	1.0 ± 12.4	−1.8 ± 11.0	0.2127	12.1 ± 8.0	10.3 ± 8.6	0.1793
Second analysis						
Number of subjects	3	44		2	37	
Oxygen uptake at VT (ml/kg/min)	12.8 ± 1.8	11.4 ± 1.7	0.1685	11.9 ± 1.0	11.1 ± 1.5	0.4774
Work rate at VT (watt)	53.3 ± 17.6	39.5 ± 11.9	0.0642	37.5 ± 3.5	29.2 ± 8.8	0.1980
Heart rate at VT (beats/min)	93.0 ± 7.2	91.0 ± 14.4	0.8183	100.5 ± 13.4	96.7 ± 12.8	0.6887

Values are means ± SD. *p* values in boldface are significant

VT ventilatory threshold

Table 5 Changes in anthropometric and physical fitness parameters in the first and second analyses in subjects without medications

	Men			Women		
	60–69	70–79	*p*	60–69	70–79	*p*
First analysis						
Number of subjects	195	34		490	50	
Height (cm)	164.8 ± 5.0	160.7 ± 6.3	**<0.0001**	152.2 ± 5.1	150.4 ± 5.5	0.0189
Body weight (kg)	65.7 ± 8.2	58.2 ± 8.8	**<0.0001**	54.5 ± 7.6	53.8 ± 7.4	0.5481
Body mass index (kg/m^2)	24.2 ± 2.6	22.5 ± 3.0	**0.0012**	23.5 ± 3.1	23.9 ± 3.5	0.4736
Abdominal circumference (cm)	85.4 ± 8.8	80.5 ± 9.4	**0.0035**	77.2 ± 8.8	80.0 ± 10.9	**0.0383**
Hip circumference (cm)	91.8 ± 4.7	88.2 ± 5.9	**0.0001**	89.9 ± 5.2	90.2 ± 4.7	0.7027
Right grip strength kg)	38.2 ± 6.2	33.4 ± 7.4	**<0.0001**	23.1 ± 4.5	22.7 ± 4.0	0.5974
Left grip strength (kg)	36.8 ± 6.3	30.6 ± 6.5	**<0.0001**	22.0 ± 4.3	21.9 ± 3.9	0.8747
Leg strength (kg)	54.7 ± 12.4	43.5 ± 11.3	**<0.0001**	36.3 ± 8.0	34.8 ± 8.1	0.1953
Leg strength per body weight	0.84 ± 0.19	0.76 ± 0.21	**0.0250**	0.67 ± 0.15	0.65 ± 0.16	0.3706
Flexibility (cm)	2.5 ± 10.5	1.0 ± 12.4	0.4562	11.6 ± 8.0	12.1 ± 8.0	0.6805
Second analysis						
Number of subjects	30	3		53	2	
Oxygen uptake at VT (ml/kg/min)	12.9 ± 1.8	12.8 ± 1.8	0.9404	11.8 ± 1.6	11.9 ± 1.0	0.9520
Work rate at VT (watt)	59.5 ± 12.5	53.3 ± 17.6	0.4342	40.5 ± 9.4	37.5 ± 3.5	0.6526
Heart rate at VT (beats/min)	97.1 ± 10.2	93.0 ± 7.2	0.5071	99.6 ± 12.6	100.5 ± 13.4	0.9253

Values are means ± SD. *p* values in boldface are significant

VT ventilatory threshold

skeletal muscle mass. It has also been well reported that there is significant loss in oxygen uptake at the ventilatory threshold (VT) –which is also considered an accurate and reliable parameter of aerobic exercise level [13]–with aging [18, 19]. Miura reported that oxygen uptake at VT was significantly correlated with age (men, $r = -0.626$; women, $r = -0.578$) in 610 Japanese [18]. Sanada et al. reported that a negative correlation was noted between oxygen uptake at VT and age in 1,463 Japanese [19]. However, there are few reports of the evaluation of physical fitness in elderly Japanese. In the previous studies noted above, the number of subjects over the age of 70 was 20 in men and 16 in women [18], and 65 in men and 13 in women [19]. Especially, there are no accurate reference data for physical fitness in Japanese subjects over the age of 70 without medications. We have previously reported on changes in maximal oxygen uptake in subjects aged 20–69 years [20]. In the present study, we evaluated anthropometric parameters, muscle strength, flexibility, and aerobic exercise levels in subjects over the age of 60. We measured anthropometric parameters, muscle strength, and flexibility in 145 men and 199 women in their 70s.

In addition, we compared parameters between subjects with and without medications. Although we evaluated VT in only 3 men and 2 women in their 70s without medications, this information gathered should serve as quite a useful database for evaluating anthropometric parameters, muscle strength, flexibility, and aerobic exercise levels in elderly Japanese subjects.

We found a difference in anthropometric parameters and muscle strength between men with and without medications in their 60s and 70s. However, in women, abdominal circumference in those in their 70s was higher than that in women in their 60s, while other parameters in women in their 70s were similar to values in those in their 60s. Sanada et al. [19] also reported that, in women, fat-free body mass in those in their 70s (41.5 ± 3.5 kg) was similar to that in women in their 60s (40.0 ± 4.4 kg), while in men, fat-free mass in those in their 70s (52.9 ± 4.1 kg) was lower than that in men in their 60s (55.3 ± 52.9 kg). Previous exercise habits, current exercise habits, and sample size may affect the results.

There are potential limitations in the present study. First, our study was a cross-sectional and not a longitudinal study. Second, the 2,106 elderly subjects, all of whom wanted to change their lifestyle, underwent measurements for this study: they were therefore more health-conscious than the average person. The 569 subjects selected in the second analysis underwent aerobic exercise testing; they were therefore more health-conscious than most of the subjects in the first analysis. Third, the small sample size, especially of subjects in their 70s without medications, might make it difficult to compare aerobic exercise levels between subjects with and without medications, and to compare these levels between subjects in their 60s and those in their 70s. In addition, the death rate in subjects aged 75–79 is higher than that in those aged 70–74 [21]. Therefore, it is well expected that there are differences in physical fitness between subjects aged 70–74 and those aged 75–79. Further large-sample-size and prospective studies are needed in elderly Japanese.

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Chair-rising and 3-min walk: A simple screening test for functional mobility

Tae-Woong Oh[1], Izumi Tabata[2], Jin-Hwan Kim[3], Tae-Hyun Lee[4], Tatsuki Naka[5]

[1]Graduate School of Health Science, Matsumoto University, Matsumoto, Japan; taewoong@matsu.ac.jp
[2]Graduate School of Sport and Health Science, Ritsumeikan University, Kusatsuki, Japan
[3]College of Physical Education, Kei-Myung University, Dae-Gu, Korea
[4]College of Martial Arts, Yong-In University, Yong-In, Korea
[5]Department of Health & Sports Science, Faculty of Health Science, Shigakkan University, Obu, Japan

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ABSTRACT

Aging induces decrease of locomotor capacity and its decrease is associated with an increased risk of falls. Several lines of evidence indicate that both change in muscle power and aerobic fitness are causative. Mobility tests are usually based on a maximal exercise stress test; however, this test is often difficult and sometimes frightening to older persons. Therefore, the objective of this study was to examine age and gender differences in 3-min walk distance test (3WDT), and time of chair-rising test (CRT) of functional mobility. 153 men and 159 women aged from 20 to 78 years were recruited as subjects of the present study. The body composition measured the height, body mass (BM), body mass index (BMI), lean tissue mass (LTM), and waist circumference (WC). The Functional mobility tests measured the peak oxygen uptake (V_{O2peak}), 3WDT, leg extension strength (LES), and times of CRT. Both in men and women, height and BMI, WC decreased and increased, respectively, with age. Height, BM, LTM, WC in men are higher than in women. We found no correlation between ages and 3WDT in women and a significant, negative correlation in men. All parameters of fitness performance were negatively correlated with age. Both in men and women, all parameters of fitness performance were positively correlated with sex. Both in men and women, V_{O2peak}, 3WDT, and LES decreased with age. All parameters of fitness performance in men are higher than in women. Both in men and women were observed for the correlation between 3WDT and V_{O2peak}, LES and CRT respectively. Although as the correlation coefficient between 3WTD and V_{O2peak}, LES and CRT were low (r = 0.28 - 0.38), an error may occur, this study shows that 3WDT and CRT test can be a feasible method of providing the information for muscle power and aerobic fitness, possibly avoiding the need for a maximal stress test.

Keywords: Peak Oxygen Uptake; 3-Min Walk Distance Test; Leg Extension Strength; Chair-Rising Test; Mobility

1. INTRODUCTION

Aging induces decrease of locomotor capacity and its decrease is associated with an increased risk of falls. Current demographic trends show that the number of older people is rapidly increasing. In fact, mobility is essential for functional independence, reduced risk of fall, and quality of life [1-3]. In older persons, disability is caused by both change in muscle power and aerobic fitness is causative. Several studies have shown that there is a decline in the ability to perform muscle power-related tests as age increases with a significant decline commencing at approximately 40 years of age. Similarly, physical performance decrease with age. These age-related changes in the performance of functional mobility measures and physiological domains are also associated with an increased risk of falls, ongoing disability and admission into residential aged care [3,4].

Mobility tests are commonly used to assess function and frailty in older persons. Mobility tests are usually based on a maximal exercise stress test; however, this test is often difficult and sometimes frightening to older persons. 3-min walk distance test and chair-rising test are low of risk. There are little data available on the age-related changes and gender differences in the performance of these tests. The development of age stratified normative data for these commonly used functional mobility tests could assist in the targeting of interventions for people who exhibit a decline in their functional status at an early stage, prior to the occurrence of falls and the onset of disability. Therefore, the aim of this study was to provide reference data and examine age and gender differences in 3-min walk distance test, and time of rising chair without using the arms. The second aim was to provide data available on the age-related changes in the performance of these tests. The information provided is relevant to new functional mobility tests in older persons.

2. METHODS

2.1. Participants

One hundred and fifty-three men and one hundred and fifty-nine women aged from 20 to 78 years (44.3 ± 14.8 years) were recruited as subjects of the present study. None of the subjects had any chronic diseases or were taking any medications that could affect the study variables. All subjects provided written informed consent according to local institute policy before the measurement of physical fitness. All subjects were classified into six groups by sex and age: 20 to 39-year-old men, 40 to 59-year-old men, 60 to 79-year-old men, 20 to 39-year-old women, 40 to 59-year-old women, and 60 to 79-year-old women. This study has been approved by the Committee on the Use of Human Research Subjects of Matsumoto University, and also performed in accordance with the ethical standards of the IJSM [5]. Participants were fully informed of the purpose and risks of participating in this investigation and signed informed consent documents prior to testing. The participants characteristics are described in **Table 1**.

2.2. Anthropometrics

The body composition measured the height, body mass (BM), body mass index (BMI), lean tissue mass (LTM), and waist circumference (WC). Height was measured to the nearest 0.1 cm using a stadiometer (YKH-23; Yagami Inc., Japan). BM, BMI, and LTM were measured using a body composition meter (BC-118E; TANITA Inc., Japan).

2.3. Functional Mobility Tests

The four tests were administered in a single session. Timed tests were measured with stopwatch with an accuracy of 0.01s.

2.4. Leg Extension Strength (LES)

LES was assessed using GT-330 (OG-giken, Japan). The individuals were seated in the chair of the dynamometer, and were stabilized with straps across the waist and thighs throughout the test. Bilateral reciprocal contractions at the knee were measured at a preset angle of 120˚. An index of strength was determined by summing peak extension torque. The average value in two times the right and left was assumed to be measurements.

2.5. Peak Oxygen Uptake (V_{O2peak})

V_{O2peak} was measured using a maximal graded exercise test (GTX) with bicycle erometers (Monark Ergomedic 828E, Sweden). The initial workload was 30 - 60 W, and the work rate was increased thereafter by 15 W·min^{-1} until subject could not maintain the required pedaling frequency (60 rpm). Heart rate (WEP-7404; NIHON KOHDEN Corp., Japan) and a rating of perceived exertion were monitored throughout the exercise. During the progressive exercise test, the expired gas of subjects was collected, and the rates of oxygen consumption and Carbon dioxide production were measured and averaged over 30-s intervals using an automated breath-by-breath gas analyzing system (Aeromonitor AE-280S; Minato Medical Science, Japan).

2.6. Chair-Rising Test (CRT)

In this test, participants were asked to rise from a standard height (43 cm) chair without armrests, ten times as fast as possible with their arms folded. Arms are crossed in front of the chest. Participants undertook the test barefoot. The time from the initial seated position to the final seated position after completing ten stands was the test measure. Two trials were to be performed. The higher value in two trials was assumed to be measurements.

Table 1. Physical characteristic of the study subjects, mean ± SD.

	All	Age 20 - 30 years	Age 40 - 50 years	Age 60 - 70 years	All	Age 20 - 30 years	Age 40 - 50 years	Age 60 - 70 years
		Men					Women	
N	153	79	44	30	159	69	54	36
Height (cm)	169.7 ± 6.7	171.0 ± 6.6	170.5 ± 6.0	164.9 ± 5.7$^{**\#}$	157.6 ± 5.6^{+++}	159.6 ± 5.1	158.4 ± 4.3	152.7 ± 5.1$^{***\#\#\#}$
BW (kg)	62.8 ± 7.8	62.2 ± 8.1	64.7 ± 6.5	61.7 ± 8.3	50.8 ± 6.1^{+++}	50.8 ± 6.2	52.3 ± 6.4	48.4 ± 4.9$^{\#}$
BMI (kg/m^2)	21.8 ± 2.2	21.2 ± 2.0	22.2 ± 1.9**	22.6 ± 2.7*	20.4 ± 2.1^{+++}	19.9 ± 2.1	20.8 ± 2.1*	20.8 ± 2.2*
MV (kg)	50.4 ± 5.0	50.7 ± 5.1	51.5 ± 4.3	48.1 ± 5.1	35.6 ± 3.1^{+++}	36.1 ± 3.2	36.3 ± 2.8	33.4 ± 2.2$^{***\#\#\#}$
WS (cm)	77.4 ± 7.2	74.6 ± 6.8	79.2 ± 5.7***	82.3 ± 7.1***	73.0 ± 6.8^{+++}	70.3 ± 5.6	74.0 ± 6.0**	76.4 ± 7.9$^{***\#}$

SD = Standard Deviation; BW = Body Weight; BMI = Body Mass Index; MV = Muscle Volume; Waist Size = WS; $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$ vs Age 20 - 30 years $^{\#\#\#}p < 0.001$, $^{\#\#}p < 0.01$, $^{\#}p < 0.05$ vs Age 40 - 50 years $^{+++}p < 0.001$ vs men.

2.7. 3-Min Walk Distance Test (3WDT)

The participants performed the 3WDT in a 50-m indoor corridor with marks every second metre on the side of the walkway. They were instructed to wear comfortable shoes. The instructions were to walk as many lengths as possible in three minutes, without running or jogging. To clarify the instructions, the participants were also told to walk as fast as possible. Information was given during the test by telling the participants how many minutes they had walked or minutes remaining. Finally, the total 3WDT was measured.

2.8. Statistical Analyses

Results are expressed as mean values with their standard errors. The statistical significance (p, 0.05) of differences was determined by 2-way ANOVA followed by a Tukey post hoc analysis. Correlations between a fitness performance and another fitness performance were assessed by Pearson's correlation coefficients (r).

3. RESULTS

The physical characteristic of the study is described in **Table 1**. Height decreased and BMI, WC increased in men, respectively, with age. Height, BM and LTM decreased and BMI and WC increased in women. All physical characteristic in men are higher than in women. **Table 2** reports the correlation between ages and functional mobility tests. All functional mobility tests, except for 3WDT in women, were negatively correlated with age (**Table 2**). **Table 3** reports the parameters of functional mobility tests of the study subjects. All parameters of functional mobility tests were positively correlated with sex. All parameters of functional mobility tests in men are higher than in women. Both in men and women were observed for the correlation between 3WDT and V_{O2peak}, LES and CRT respectively. **Figure 1** reports the relationship between 3WDT and V_{O2peak} in the men (n = 153) and women (n = 159). Both in men and women, 3WTD was correlated with V_{O2peak} (r = 0.31 and 0.31, respectively; $p < 0.0001$). **Figure 2** reports the relationship between LES and CRT in the men (n = 153) and women (n = 159). Both in men and women, LES was correlated with CRT (r = 0.38 and 0.28, respectively; $p < 0.001$).

4. DISCUSSION

3WDT and CRT is the simplest test of the V_{O2peak} test and leg strength, respectively. This study adds to the accumulating literature investigating the dynamic relations between body compositions and the functional mobility test in the elderly.

Body composition varies according to age, sex, and race. Older adults tend to lose fat-free mass and gain fat mass. WC is a reliable marker of mortality in older adults [6-8] and muscle mass, as represented by lean mass, is associated with survival. In the present study, height, BMI and WC were decreased and increased, respectively, with age in men and women. Moreover, Height, BM, LTM and WC in men is higher than in women.

The Functional mobility tests measured the V_{O2peak}, 3WDT, LES, and CRT. The study findings revealed significant age-related differences in all functional mobility tests examined. These findings confirm those of previous studies and indicate that when compared with young people, older people exhibit slower comfortable walking speed [5,9], reduced ability to quickly rise from a chair [3,10]. These age-related differences in functional mobility have been attributed to impaired sensorimotor function [11,12], in particular reduced lower extremity strength and power [13-15], but also increased fear of falling [8] and reduced aerobic capacity [16].

Table 2. Correlation of the variables of interest with age.

	CRT (sec)	3WDT (m)	LE (n - m)	V_{O2max} (ml/kg/min)
Women	0.19^{*}	−0.12	-0.32^{**}	-0.49^{**}
Men	0.42^{**}	-0.32^{**}	-0.32^{**}	-0.51^{**}

CRT = chair-rising test; 3WDT = 3-min walk distance test; LE = leg extension; V_{O2max} = maximum oxygen uptake; Pearsons correlation coefficients. $^{**}p < 0.001$, $^{*}p < 0.05$.

Table 3. Parameters of fitness performance of the study subjects, mean ± SD.

	All	Age 20 - 30 years	Age 40 - 50 years	Age 60 - 70 years	All	Age 20 - 30 years	Age 40 - 50 years	Age 60 - 70 years
	Men				Women			
N	153	79	44	30	159	69	54	36
V_{O2max} (ml/kg/min)	36.7 ± 9.1	40.7 ± 8.1	$35.0 \pm 7.7^{***}$	$28.6 \pm 6.9^{***\#\#\#}$	$27.8 \pm 6.4^{+++}$	30.6 ± 6.5	$27.4 \pm 5.3^{**}$	$23.0 \pm 4.4^{***\#\#\#}$
CRT (sec)	10.2 ± 2.4	9.5 ± 1.8	9.6 ± 1.7	$12.8 \pm 3.1^{***\#\#\#}$	$11.1 \pm 2.5^{+++}$	10.7 ± 2.7	11.2 ± 2.3	11.6 ± 2.5
3WDT (m)	390.0 ± 58.7	403.3 ± 58.9	390.7 ± 38.9	$353.7 \pm 68.0^{***\#\#}$	$350.6 \pm 34.1^{+++}$	351.1 ± 34.6	358.2 ± 31.6	$338.4 \pm 34.4^{\#}$
LE (n - m)	630.1 ± 137.0	669.3 ± 130.8	622.8 ± 136.5	$537.7 \pm 108.3^{***\#\#}$	$409.2 \pm 106.3^{+++}$	430.1 ± 118.8	420.3 ± 91.1	$352.6 \pm 81.7^{***\#\#}$

SD = Standard Deviation; CRT = chair-rising test; 3WDT = 3-min walk distance test; LE = leg extension; V_{O2max} = maximum oxygen uptake.

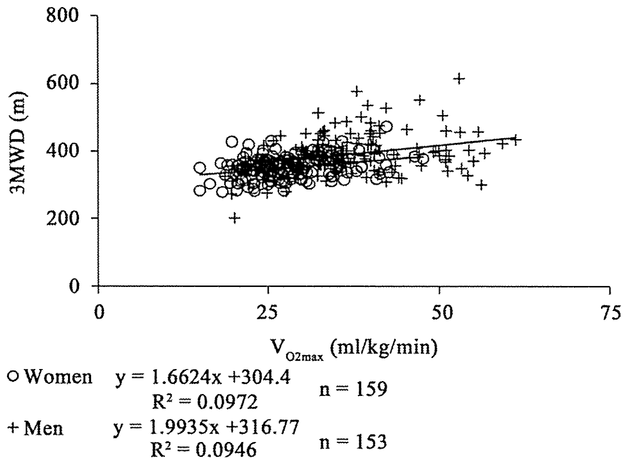

Figure 1. Relationship between 3WTD and V_{O2max} in the men (n = 153) and women (n = 159). The 3MWD was to walk as many lengths as possible in three minutes. V_{O2max} was to until subject could not maintain the required pedaling frequency (60 rpm).

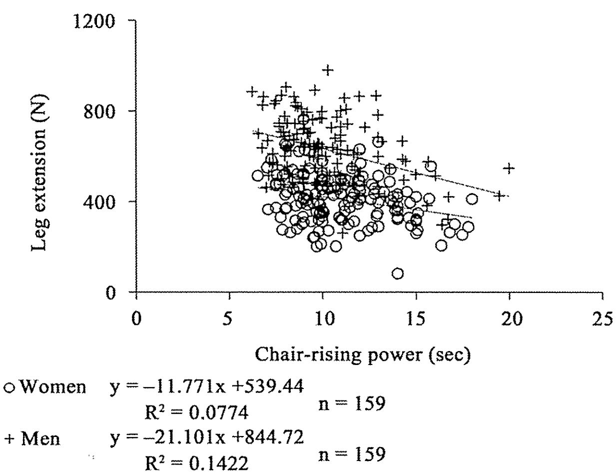

Figure 2. Relationship between LE and CRT in the men (n = 153) and women (n = 159). The LE was to the highest in four times in total the right and left two times value. Chair-rising times from the initial seated position to the final seated position after completing ten stands were the test measure.

A remarkable decline, however, was observed in the performance variables (muscle strength and aerobic capacity) assessed by the CRT and by 3WDT. The CRT was measured power during an activity which involves raising the centre of gravity. Several studies have found that performance in the CRT is a strong predictor of incident disability, mortality, falls, hospitalization and health care resources consumption. Hence, CRT can be regarded as an indicator of physical performance at old age. In the present study, both in men and women were observed for the correlation between 3WDT and V_{O2peak}, LES and CRT respectively, and the fact that this test can be a feasible method of providing the information for muscle power and aerobic fitness, possibly avoiding the need for a maximal stress test.

Significant correlations among all the functional mobility tests in the older group indicate that older adults who performed poorly in one test were likely to perform poorly in all the other tests. The results from the present study, the functional mobility tests of 3WDT and CRT were found to give an idea of the physical decline with age in fit elderly without any maximal exercise stress.

In conclusion, first, this study provides significant age-related differences in performance were found in tests of coordinated the V_{O2peak}, 3WDT, LES, and CRT, with older women performing worse than older men in all tests. Secondly, this study shows that 3WDT and CRT can be a feasible method of providing the information for muscle power and aerobic fitness, possibly avoiding the need for a maximal stress test.

Limit

As the correlation coefficient between 3WTD and V_{O2peak}, LES and CRT were low (r = 0.28 - 0.38), an error may occur. Accordingly, this study shows that 3WDT and CRT as estimate method for aerobic fitness and muscle power can be a feasible, if we measure many people as method briefly and in safety.

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