Table 1. Incidence of MuSK-MG in Seven Mouse Strains

Strain	Total number of mice	MG symptoms (no. of mice)			Incidence (%)
		No symptoms	>10% CMAP decrement		
			<20% weight loss	>20% weight loss	
A/WySnJ	18	0	0	18	100
A/J	5	0	0	5	100
DBA/2	5	0	1	4	100
FVB/N	5	0	0	5	100
B10.A-*H2*^{a}	4	0	4	0	100
BALB/c	4	1	1	2	75.0
C57BL/6	11	6	4	1	45.5

CMAP, compound muscle action potential.

(*Tnfrsf13c*)]. To determine whether the presence of this mutant allele affects the onset of MuSK-EAMG regardless of C5 deficiency, several strains of mice carrying no *Bcmd-1* mutant allele (A/J, DBA/2, FVB/N), but exhibiting the same C5 deficiency as A/WySnJ,[19] were injected with MuSK protein in the same manner and on the same schedule. Almost all of the mice tested exhibited a marked decrease in body weight in addition to impairment of neuromuscular transmission, indicating that a severe form of MuSK-EAMG is inducible in other C5-deficient strains at high incidence (Table 1). Of note, these mice were more sensitive to the onset of MuSK-EAMG than were complement-sufficient mice, such as B10.A-*H2*^{a}, BALB/c, and C57BL/6. Notably, approximately one half of the C57BL/6 mice examined exhibited neither significant weight loss nor neuromuscular transmission defect, despite receiving three injections (Table 1). These results imply that C5-deficient strains may share the genetic background that confers susceptibility to severe MuSK-EAMG.

To validate the results obtained using A/WySnJ mice, genetically engineered C3-deficient mice were used to induce MuSK-EAMG.[35] Although two out of five MuSK-injected mice exhibited moderate (<20%) weight reduction 2 weeks after the third injection (see Supplemental Figure S1 at *http://ajp.amjpathol.org*), these mice exhibited a significant decrease in muscle strength, compared with controls. In addition, all MuSK-injected mice exhibited a decremental CMAP amplitude response after repetitive nerve stimulation, indicating that neuromuscular transmission was impaired (see Supplemental Figure S1, D and E, at *http://ajp.amjpathol.org*). In whole-mount staining of soleus muscles from MuSK-injected mice, the pretzel-like structures of AChR clusters were totally disassembled, and axon sprouting was observed in many NMJs (see Supplemental Figure S1F at *http://ajp.amjpathol.org*). Taken together, these results are consistent with those obtained from A/WySnJ mice, confirming that complement activation is dispensable for the onset of MuSK-EAMG.

MuSK Abs Impair AChE Function at NMJs

AChE inhibitors are an effective for treatment of AChR-MG symptoms, but may cause muscle cramps, fasciculation, dysphagia, and respiratory insufficiency in MuSK-MG patients.[14,36] Therefore, to investigate the effect of AChE-inhibition on recovery of neuromuscular transmission, we administered the AChE inhibitor neostigmine to MuSK-injected mice, and recorded CMAPs by repetitive nerve stimulation before and 20 minutes after neostigmine administration (37.5 μg/kg). Although all neostigmine-treated, MuSK-injected mice (n = 6) exhibited a significant reversal in CMAP decline, only one mouse recovered CMAP to within 10% of the decline (9.2%), which is considered a normal response (Figure 6, A and B). Of note, in two neostigmine-treated, MuSK-injected mice, we recorded an abnormal EMG pattern 5 to 20 ms after the CMAP peak in the first trace in a stimulation series, as is observed in MuSK-MG patients after AChE inhibitor treatment (Figure 6C).[37,38] Furthermore, another abnormal EMG pattern related to a congenital myasthenic syndrome caused by an AChE deficiency was recorded in a separate MuSK-injected mouse (Figure 6D).[39] Repetitive nerve stimulation at 3 Hz elicited a second CMAP with smaller amplitude than the first, a moderate decline of the primary CMAP, and a faster decline of the secondary CMAP. We did not observe abnormal EMG patterns after neostigmine injection in control mice (data not shown). These observations indicate abnormal sensitivities to ACh at endplates after MuSK injection, and suggest that the pathological conditions of patients with neuromuscular disorders could be reproduced in MuSK-injected mice.

Given that abnormal electrophysiological signs have been observed in MuSK-MG patients administered an overdose of AChE inhibitors or in patients with congenital myasthenia with AChE deficiency,[38,39] we postulated that AChE deficiency relative to ACh activity at NMJs might be the cause of these abnormalities. We therefore examined the expression of AChE and the AChE-anchoring protein ColQ, which may bind to MuSK and promote the accumulation of AChE at the postsynaptic membrane.[40,41] Using BTx and antibodies against AChE and ColQ, we triple-labeled soleus muscle whole mounts from MuSK-injected and control mice. The intensities of both AChE and ColQ expression at NMJs in MuSK-injected mice were reduced (Figure 6E), and the area of AChE expression was decreased significantly in MuSK-injected mice (n = 104 NMJs in 3 mice), compared with control mice (n = 91 NMJs in 3 mice) (Figure 6, E and F).

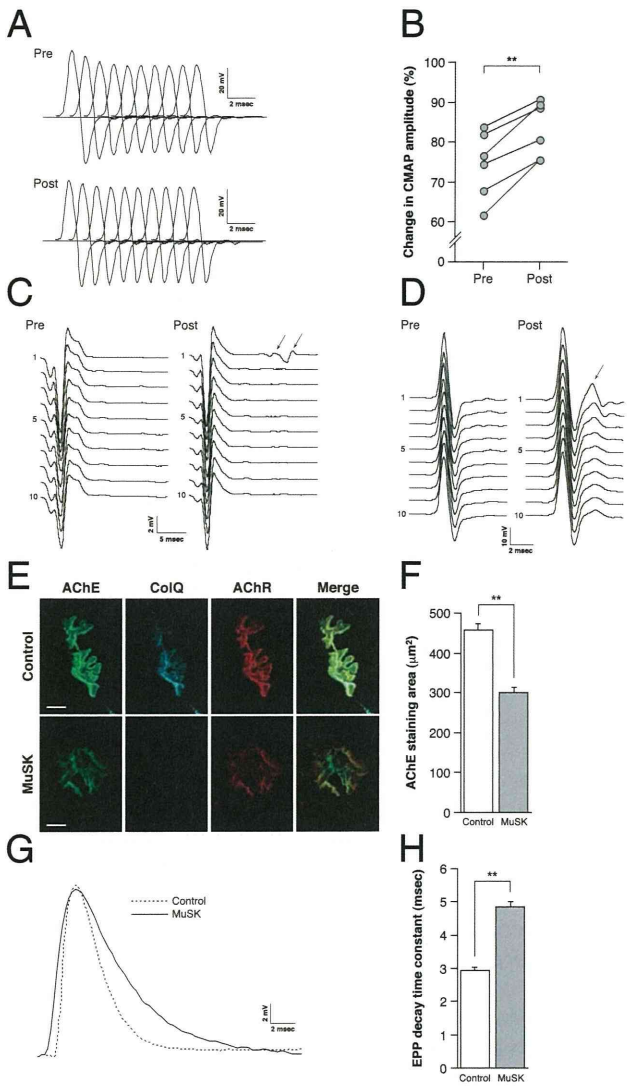

Figure 6. Impairment of AChE function in MuSK-injected mice. **A:** Representative EMG traces before and after neostigmine treatment. **B:** Neostigmine induced a significant reversal of CMAP decrement, ranging from 23.4% to 54.1% (mean, 36.4 ± 4.8%). $^{**}P < 0.01$ (paired *t*-test). **C** and **D:** Abnormal EMG traces evoked by neostigmine treatment. After neostigmine treatment, disturbance of baseline (**arrows**) after CMAP emerged in the first trace only (**C**), and a second CMAP (**arrow**) emerged, with smaller amplitude than the first, and then gradually disappeared (**D**). **E:** Levels of antibody staining for AChE (green), ColQ (cyan), and Alexa Fluor 647-BTx labeling of AChRs (red) were reduced in soleus muscles of MuSK-injected mice, compared with controls. Scale bars: 10 μm. **F:** The average AChE staining area decreased to 65.8% of control values in MuSK-injected mice; ≥30 NMJs from each mouse (n = 3 mice/group) were quantified. **G:** Representative EPP traces from control and MuSK-injected mouse diaphragms. In EPP traces with equivalent amplitudes, MuSK-injected mice exhibited longer EPP decay time constants (τ = 5.0 ms), compared with controls (τ = 2.6 ms). **H:** The mean EPP τ of MuSK-injected mice was 164% that of control mice. Data are expressed as means ± SEM from ≥10 NMJs of each mouse (n = 3 mice/group). $^{**}P < 0.01$ versus control mice (*t*-test).

Furthermore, we investigated the impairment of AChE function by examining the rate of EPP decay, the EPP decay time constant (τ), using intracellular recordings as described above.[42] In traces with similar amplitudes, the falling phase of the EPP of MuSK-injected mice was not as steep as that of controls (Figure 6G), and the mean EPP τ of MuSK-injected mice (4.85 ± 0.16 ms, n = 30 NMJs in 3 mice) was longer than that of controls (2.95 ± 0.07 ms, n = 53 NMJs in 3 mice) (Figure 6H), indicating that the AChE at NMJs in MuSK-injected mice could not hydrolyze ACh rapidly, leading to prolonged ACh action. Taken together, our results demonstrate that MuSK is required for the binding of AChE clusters to ColQ at the NMJ postsynaptic membrane *in vivo* and that MuSK Abs interfered with AChE function, leading to hypersensitivity to ACh after neostigmine treatment.

Discussion

In the present study, we investigated the pathophysiology of MuSK-MG using a new animal model in which 100% of mice synchronously develop MG after MuSK protein injection. In addition, we demonstrated the pathogenicity of MuSK Abs and found novel roles for MuSK activity at mature NMJs.

We previously demonstrated the pathogenicity of MuSK Abs via active immunization of rabbits with recombinant soluble MuSK protein.[7] In addition, EAMG induced by inoculation of MuSK protein has also been successfully established in mice.[23,43] Furthermore, when passive transfer of human MuSK Abs from MG patients into mice caused severe muscle weakness,[8] and EMG results were compatible with a diagnosis of MG, the pathogenicity of MuSK Abs was confirmed. Although it has been postulated that the human IgG4 subclass of autoantibodies causes MuSK-MG without complement activation, previous pathophysiological studies did not include complement-deficient animals.[9,10,44]

The A/WySnJ mouse strain cannot generate the membrane attack complex, which is the cytolytic end product of the complement cascade, because of mutations in the complement component 5 gene (*C5*).[19] Thus, the use of this strain allowed us to completely eliminate the role of complement activation on the onset of MG and to analyze non-complement-mediated effects. Previously, a high incidence of EAMG after MuSK immunization was also observed in A/J mice, which exhibit the same C5 deficiency as A/WySnJ mice.[23] We demonstrated that not only A/J but also other C5-deficient strains exhibited a severe form of MuSK-EAMG at a high incidence, as observed in the A/WySnJ strain. Complement-mediated damage to postsynaptic membranes is considered to be the major pathogenic mechanism in AChR-MG, because complement-deficient mice are resistant to EAMG induced by AChR immunization.[20,21] However, our results demonstrate that complement activation is not necessary for the onset of MuSK-MG, and provide insight into the mechanism by which the IgG4 subclass of MuSK Abs causes human MG.[9,10]

The IgG4 subclass of MuSK Abs observed in MG patients may have a functionally monovalent antigen-binding site, because IgG4 exchanges Fab arms with non-pathogenic IgG4 *in vivo*.[45] Therefore, this particular IgG4 may not efficiently reduce the number of MuSK molecules on the surface of postsynaptic membranes by invoking a cross-linking mechanism requiring divalent antibodies. Although no dynamic exchange of the IgG-Fab arm is found in mouse IgG subclasses, we demonstrated *in*

vitro that the monovalent Fab fragments of MuSK-IgG inhibited MuSK signaling and AChR clustering. Therefore, MuSK antibody binding could directly inhibit MuSK function required for the maintenance of mature NMJs, as well as internalization of MuSK molecules from the plasma membrane.[7,46]

In the present study, we demonstrated that MuSK is required for maintenance of mature NMJ structure and function. Our complement-deficient mouse model exhibited a loss of AChR expression, as well as a reduction in the size of motor terminals apposing AChR clusters at NMJs. These changes were consistent with those observed in complement-sufficient animals that developed MuSK-EAMG[7,8,23,43] and in mice bearing the MuSK mutations observed in congenital myasthenic syndrome.[47,48] Furthermore, our ultrastructural studies indicate a significant loss of complexity in convoluted postsynaptic structures (eg, the flattening of synaptic gutters), as well as a decrease in the number of slit-like junctional folds at postsynaptic membranes.

In addition to morphological defects, our model exhibited functional defects at presynapses, including lower levels of transmitter release from nerve terminals. In clinical studies, low levels of presynaptic ACh release were found at NMJs of a MuSK-MG patient using *in vitro* electrophysiology, which is consistent with our results.[49,50] It has been shown that AChR-MG, which is caused exclusively by postsynaptic defects, results in an increase in quantal content in both patients and EAMG model animals, suggesting the involvement of a compensatory retrograde signaling mechanism between postsynaptic and presynaptic sites.[51,52] Therefore, the decrease in quantal content in MuSK-MG might imply dysfunction of this compensatory mechanism, suggesting that MuSK mediates retrograde signaling involving transmitter release from nerve terminals. In this regard, mice that carry an inactivated form of the gene encoding muscle-specific β-catenin, a signaling protein downstream of disheveled (Dvl), or laminin $\beta 2$, also have morphological defects both presynaptically and postsynaptically.[31,32] These data point to the important roles these molecules play in NMJ formation. Furthermore, both spontaneous and evoked ACh release from nerve terminals are reduced by abnormalities in the vesicle release machinery or reductions in the number of release sites. Therefore, we cannot exclude the possibility that the defects observed in our EAMG model might result from impairment of the presynaptic component itself, via dysfunction of MuSK (although it is generally assumed that the reductions observed in presynaptic and postsynaptic areas are related to diminished ACh release[17]). Our data suggest that MuSK plays a role in the maintenance of presynaptic function at NMJs through retrograde signaling.

We demonstrated that MuSK is required for proper AChE function at NMJs and determined the mechanism by which AChE inhibitor treatment exacerbates MuSK-MG symptoms. It is clear that MuSK participates in the accumulation of AChE in the synaptic basal lamina of NMJs, possibly by forming complexes with ColQ, as postulated previously based on *in vitro* experiments.[41] MuSK might act as a scaffold molecule, anchoring those complexes to the synaptic basal lamina. Additionally, MuSK signaling may be required for the highly localized expression of AChE mRNA at mature NMJs.[53,54] Accumulated AChE in the synaptic cleft may tightly limit both the temporal and spatial extent of cholinergic neurotransmission by rapid cleavage of ACh at the basal lamina. However, inhibition of MuSK by MuSK Abs decreased levels of AChE at the basal lamina and impaired its function, as shown by the prolonged EPP time constant we observed. If AChE inhibitors were to be administered in these conditions, the rebinding to AChRs by an excess of ACh existing at the synaptic cleft could provoke cholinergic hypersensitivity (recorded as an abnormal EMG pattern) and so could eventually lead to cholinergic crises.

Clinically, MuSK-MG patients tend to have severely weak, atrophied muscles and are more refractory to treatment than AChR-MG patients. These differences in clinical symptoms between MuSK-MG and AChR-MG could result from the distinct pathogenic mechanisms involved in these two types of MG. Overall, our results indicate that MuSK plays indispensable roles in the structural and functional maintenance of mature NMJs, and that disruption of MuSK function by specific autoantibodies causes MG (Figure 7). One of the main signals that cause advancement from presynapse to postsynapse stages is the agrin/MuSK pathway, but the retrograde signals regulated by MuSK are still elusive. Although fibroblast growth factors, laminin $\beta 2$, and collagen α(IV) chains act as muscle-derived organizers of presynaptic differentiation, whether they act in conjunction with MuSK signaling is uncertain.[55,56] Our model of MuSK-MG did not exhibit immune complex-mediated damage to tissue that would otherwise participate in this interaction. Our model thus provides a valuable platform from which to evaluate the role of MuSK signaling in NMJ maintenance and the immune mechanisms and pathophysiology of MuSK-MG.

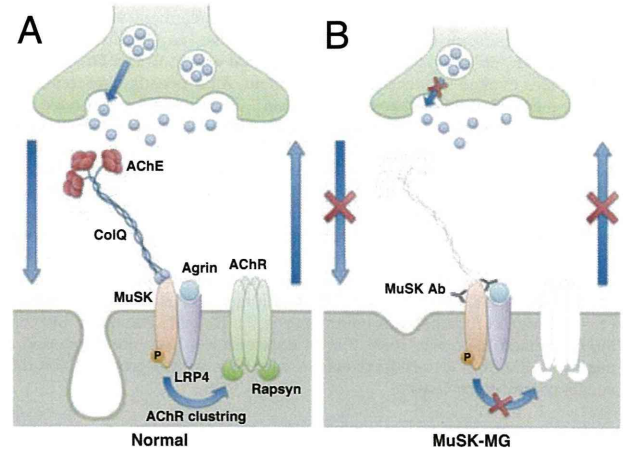

Figure 7. Pathogenic mechanism of MG induced by MuSK Abs. **A:** In normal NMJs, agrin binds to LRP4 to activate MuSK. MuSK regulates the maintenance of both presynaptic and postsynaptic structures and functions bidirectionally. **B:** MuSK Abs bind to the ectodomain of MuSK, causing MuSK degradation by antigenic modulation and/or direct inhibition of MuSK function. This inhibits MuSK, resulting in structural disruptions (eg, dispersal of AChR clusters, loss of synaptic folds, and degeneration of nerve terminals) and functional abnormalities (eg, decrease in ACh release) that eventually lead to MG. Furthermore, the decreased levels of AChE, which is anchored to MuSK by ColQ, can induce a cholinergic crisis mediated by AChE inhibitors, exacerbating the symptoms of MG.

As a result, this model could be instrumental in the development of effective medications for this group of devastating diseases.

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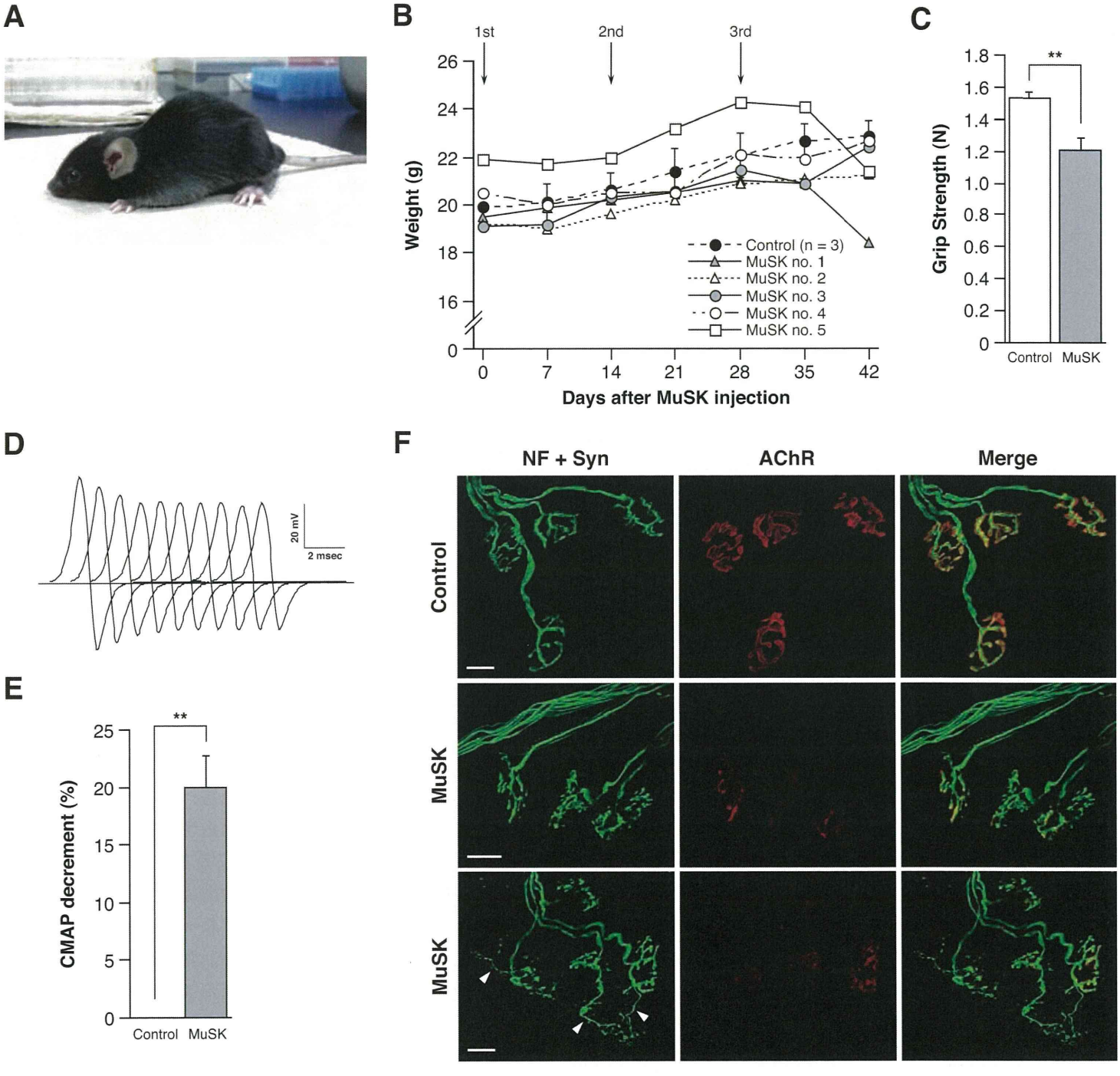

Supplemental Figure S1.

神経内科，75(6)：584-588，2011

特集 サルコペニア

サルコペニアの発症機序*

重本和宏**／森　秀一**／宮崎　剛**／久保幸穂**

Key Words : sarcopenia, satellite cell, neuromuscular junction, retrograde signal, motor neuron

はじめに

運動器障害によって治療や介護を要する状態（運動器不安定症，ロコモティブシンドロームと称される）の中で，サルコペニア（加齢性筋肉減少症）は高齢者のactivity of daily living（ADL）とquality of life（QOL）を損う主要な原因である．すでに超高齢社会を迎えているわが国だけでなく，欧米においてもサルコペニアが注目されている．サルコペニアの特徴は加齢による筋肉量低下と筋力低下であるが[1]，実際に臨床や介護現場で有効に役立てることができる定義と診断基準のいずれについても確立されていないのが現状である．さらに，サルコペニアは遺伝因子と環境要因に加えて，エピジェネティックの変化など多様な老化促進因子が長時間重なって起きるため，その病態とメカニズムを解明することを困難にしている．サルコペニアは，さまざまな原因により誘発されると終末像と考えられるが，その原因についてはほとんどわかっていない．本稿ではこのような現状を踏まえて，これまでの成因メカニズムの研究について紹介する．

サルコペニアの定義

サルコペニアの定義はまだ定まっていないのが現状であると述べた．しかし，サルコペニアの成因について議論する前に，その定義について筆者の考え方を明確にしておく必要がある．

2010年に世界に先駆けてサルコペニアの定義がEuropa Consensusとして発表された[2]．筋量と筋力，および歩行速度（運動能力）の三つを指標にして，それぞれを組み合わせることでサルコペニアを診断するとしている．これらの指標はJanssenの疫学研究の結果を基にしているが[1]，さまざまな原因によって生じるサルコペニアを早期に診断して予防するために真に有効かどうか，すでに疑問視する考え方もあり，今後も継続して検証する必要がある．それから，神経筋疾患の除外診断は当然必要である．たとえば高齢社会を背景に重症筋無力症の患者数が，18年前の全国調査に比べ総数で2.5倍増加していることが2006年に実施された厚生労働省の免疫性神経疾患に関する調査で明らかになった．高齢者は，眼瞼下垂，複視，構音障害，嚥下困難を含む筋力低下などの重症筋無力症に特徴的な症状が，若年者に比べ見過ごされがちになる．その他の神経筋疾患についても，高齢化に伴い増加しているにもかかわらず見過ごされている可能性が十分にある．サルコペニアから神経筋疾患

* Current understanding of the cellular and molecular mechanisms underlying sarcopenia.

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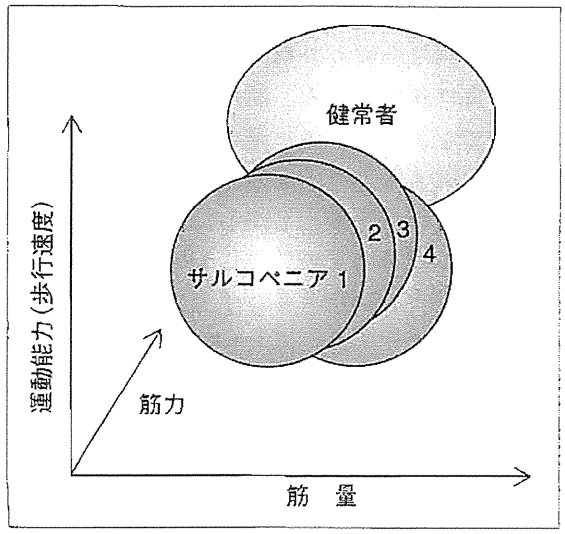

図1　サルコペニアの定義と診断
サルコペニアはさまざまな原因によって起きるが，それらを筋量，筋力および歩行速度の組み合わせで診断する意義については，今後の検討が必要である．

は除外しなくてはならない．

現在，認知症は多くの病型に分類することができるが，サルコペニアも多様な原因により筋萎縮へ収束する病態の集合群であり，まだ単一の概念として捉えているだけであると考えられる（図1）．加えて，高齢者がなんらかの原因で急性期の運動機能障害を抱えてしまうと，リハビリによる機能回復の程度は個人差が大きく困難なケースも多くなる．明らかな原因疾患（悪性腫瘍，重篤な感染症，脳血管障害，認知症）による筋肉喪失（cachexia）とサルコペニアの違いの明確な定義はないが，cachexiaのケースで基礎疾患が改善された後のリハビリによる回復力の違いは，サルコペニアの成因と関連すると考えられる．サルコペニアの病態を知る上で，病理学的データの蓄積と解析が必要であるが，認知症のような体系的な研究はこれまでほとんど行われていなかったと思われる．

以上のことから，現状では単純にサルコペニアの定義を述べることは難しいが，ここではサルコペニアを老化による筋の予備能力と回復力の喪失という観点で考えてみたい．

サルコペニアの三つの成因メカニズム

サルコペニアに関するこれまでの研究から，

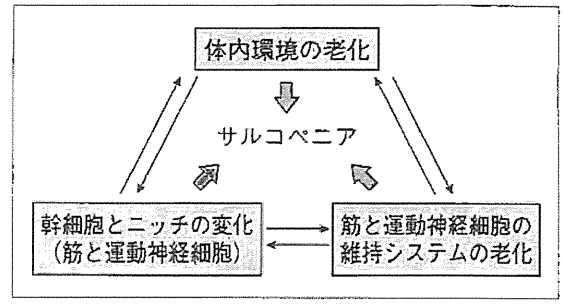

図2　サルコペニアのメカニズム
環境要因，遺伝因子，エピジェネティックの変化で誘発される3種類の経路．

加齢による筋の老化促進の要因は以下の3種類に分類することができる（図2）．体内環境全体の変化（免疫・炎症，ホルモン，代謝・栄養状態），そして，幹細胞（サテライト細胞）とそれを維持する微小環境（ニッチ）の老化，さらに筋と運動神経細胞（中枢神経）の相互作用による維持システムの老化である．これらの原因が互いに影響し合うことでサルコペニアが進行すると考えられる．

1．免疫・炎症性変化と代謝変換

加齢に伴い体内の環境は変化するが，その一つとして生体内において慢性炎症状態が生ずることにより老化現象が顕在化する可能性が提案されている[3]．また，最近では筋からも炎症性サイトカインを分泌することが明らかにされている（muscleから分泌されるcytokineをmyokineともいう）．運動習慣は筋において，遺伝子転写補助因子であるperoxisome-proliferator-activated receptor-γ coactivator-1α（PGC1α）の発現を増強するが，PGC1αはIL-6, TNF-αなどの炎症サイトカインの発現を抑制する[4]．また，サルコペニアの原因についてはミトコンドリア機能との関連が古くから指摘されているが，PGC1αはミトコンドリアの機能を正に調節する（図3）．興味深いことにPGC1αを筋に強制的に発現させたトランスジェニックマウスは，高齢になっても筋の運動機能が保たれサルコペニアを抑制し，さらに寿命も顕著に長くなるという[4]．炎症性サイトカインは脂肪細胞からも分泌されるが，サルコペニアでは筋萎縮に伴い筋肉内の脂肪組織も増大することが指摘されている（sarcopenic obesity）．

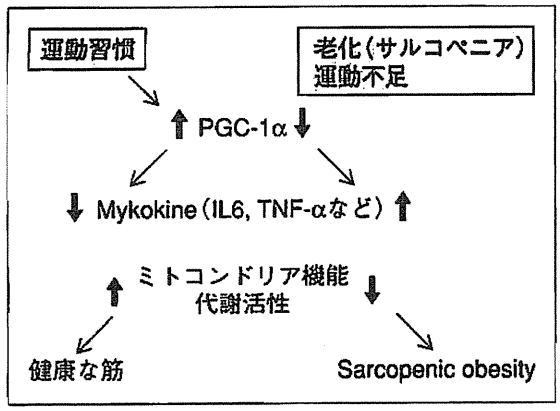

図3　サルコペニアとPGC-1αの関係を示す模式図
加齢とともに筋肉のPGC-1αの発現が減少して，筋から炎症性サイトカイン(myokine)の産生が増加する．また，筋のミトコンドリア機能が弱まり，萎縮した筋の代わりに脂肪組織が増える(sarcopenic obesity)．インシュリン感受性も減弱する．運動習慣はPGC-1αの発現を増強することで逆に作用する．

それでは，実際に高齢者においてIL6とTNF-αの発現はどうなっているであろうか．Visserらは，運動障害のない健康な米国人の高齢者(70～79歳)男女3,075人(白人とアフリカ系米国人)について横断的研究で高IL-6およびTNF-αが高齢者の筋量と筋力の低下と相関しているとしている[5]．さらに，Schaapらは炎症性マーカー(IL-6, TNF-α, CRP, 可溶性IL-6受容体とTNF-α受容体)と筋力(握力と膝伸展筋力)および筋量(CT)の変化との相関を5年間の縦断的研究で解析した[6]．筋量の減少を，体重減少を考慮に入れ補正した結果，TNF-αと可溶性TNF-α受容体の高値が筋量および筋力低下と相関があるとしている．調査開始時点では運動障害のない高齢者を選んでいるが，期間中に死亡した対象者は除外されている．

サルコペニアと慢性炎症の因果関係についてはまだよくわかっていないが，老化による筋萎縮の過程で遅筋線維から速筋線維への質的変換が起きることが20年以上も前に組織学的研究から示されている．遅筋はミトコンドリアによる代謝が主であり，一方で速筋は解糖系が優位であることから，老化による筋の質的変化は代謝変換を伴っていると考えられる．前述のPGC1αは，この代謝変化と炎症性サイトカインの分泌調整の両方に関係することから，生活習慣病とサルコペニアとの関連性も提唱されており，そのメカニズムに関する研究が注目される．

2．栄養状態とホルモン変化

通常，筋は合成と分解のバランスが保たれることで定常状態にある．筋は栄養飢餓状態において重要なエネルーギー源になることから，食生活に起因する慢性的な栄養不足はサルコペニアの成因になりうる．しかし，内在性の筋合成障害とサルコペニアの関係については，対象者の選定や測定方法がさまざまで定まった見解がないのが現状である．ビタミンDとサルコペニアに関する疫学的調査に関しても，相反する結果がこれまで報告されている．Foleyらは腎機能の低下がサルコペニアの出現と相関すると報告しているが，多変量解析を行ったところGFR低下に加え加齢，低所得，肥満，運動不足，栄養の偏り，高カルシウム血症，ビタミンDの低摂取，高拡張期血圧およびインスリン抵抗性がサルコペニアと関連していた[7]．ビタミンDは老化によるさまざまな身体機能障害とも関係があるので，サルコペニアよりはfrailty(老化による虚弱状態の概念)との関連を考えた方が良さそうである．高齢者の男性のテストステロンの減少とサルコペニアの関連が報告されている[8]．また，女性では閉経後のエストロゲンの減少が筋肉に発現するエストロゲン受容体を介して筋力と筋量の減少を誘発すると考えられているが，メカニズムは明らかにされていない[9]．Insulin-like growth factor1(IGF-1)は，蛋白合成と筋サテライト細胞を活性化して筋肉の分化成熟，維持，再生，肥大をもたらす．成長ホルモンで誘導されるIGF-1が加齢とともに減少して筋量が減少すると考えられているが，サルコペニアとの因果関係は不明である[10]．上記のホルモンは確かに筋の維持に重要な役割を果たしているが，ビタミンDと同様に多臓器に対して作用する．

3．サテライト細胞とニッチの老化

筋の幹細胞はサテライト細胞と呼ばれているが，高齢マウスを使った実験からサテライト細胞の機能低下と筋の線維化を促進する新しい体内環境因子としてWntが関与することが報告された[11][12]．Wntは細胞間シグナルを行う分泌蛋白で胚発生やガンにかかわることが知られているが，Klothoという蛋白がWntと結合することで細胞老化を抑制すると報告した．また，Conboyらは高

齢マウスと若いマウスの2匹の血管を縫合して血液循環を同じにして(parabiotic pairings)，筋に損傷を与えてその再生能を調べたところ，若いマウスとつながった高齢マウスでは筋再生能が増強し，逆に若年マウスでは筋再生能が低下して筋の線維化が進んだ[13]．その促進因子としてWntを同定した．Wntは肺や肝臓の線維化の促進因子としても報告されている．Wntとその抑制因子は，本来局所的なシグナル伝達分子として知られており，体内環境の老化因子としての作用は新しい見方である．

高齢による筋の再生能の低下の原因が上記の体内環境以外に，サテライト細胞自身やニッチの老化が関係することが報告されている．サテライト細胞のニッチとしては，周囲の筋細胞，結合組織，細胞外マトリックスなどがあげられる．高齢マウス由来のサテライト細胞は若いマウスの筋に移植すると十分な再生能を示す．したがって，前項で述べたWntに加えて，ニッチの加齢による変化がサテライト細胞に影響している可能性が考えられる．Conboyらは若いマウス由来のサテライト細胞を，高齢マウス由来の筋と共培養すると再生能が顕著に抑制することを示した．そして，高齢マウスの筋の基底膜にTGF-βが増加していること，また，TGF-βはサテライト細胞の増殖を抑制し，さらにサテライト細胞の増殖刺激に必要なNotchシグナルが減少していることを示した[13]．前に紹介したWntによる体内環境の変化とニッチとの関係は不明であるが，結合組織の増殖を介している可能が考えられる．

最近，筋細胞とはまったく異なる系譜のPDGFα陽性の脂肪前駆細胞が筋に存在することが明らかにされた[14][15]．この前駆細胞は通常は筋サテライト細胞の分化を抑制しているが，筋再生が必要なときは増殖して，おそらくIL6を介して筋サテライト細胞の分化を誘導すると考えられる[16]．

一方で，筋損傷が引き金となって前駆細胞が脂肪細胞へ分化すると筋サテライト細胞の分化を抑制するようになる．加齢変化と筋損傷，およびsarcopenic obesityのメカニズムとの関連で注目される．

4．筋と運動神経の相互作用による維持メカニズム

もともと健常筋には萎縮へと向かうカスケードが常在している．若い健常人であっても骨折などで筋活動が停止すると，2週間以内で急速に筋萎縮に至る．適切な運動習慣により，運動神経線維と筋のつなぎ目である神経筋シナプスを介した筋と運動神経の相互作用が，結果的に筋の合成と分解のバランスを保つことで筋萎縮カスケードに拮抗することができる．また，前述したサルコペニアの特徴である筋の遅筋化は，運動神経細胞により誘導されると考えられている．速筋線維を支配するシナプスは遅筋よりも選択的な脆弱性があるため，近傍の遅筋線維を支配するシナプスから代償性に伸張した運動神経終末で再神経支配された時に，速筋線維から遅筋線維へと転換すると考えられている．そして，再支配を受けることができなかった筋線維は萎縮する．しかしながら，筋と運動神経のシナプスを介した相互作用の分子機構は，いまだよくわかっていない．特に筋から運動神経への逆行性シグナルはさまざまな機能分子が予想され，たとえばシナプスの形態と機能を維持するために必要なものや，運動神経細胞に対する標的由来神経栄養因子として働いている分子もあり，筋萎縮性疾患の病因解明と治療への応用が期待される．これまで同定されたNGFファミリーやGDNFなど，神経栄養因子の運動神経細胞に対して期待された臨床治療効果は限定的であるのが現状である．老化に伴うなんらかの原因により，運動神経と筋の相互作用維持メカニズムが阻害されると筋萎縮が誘導されると考えられる[17]．運動神経細胞の脱落とサルコペニアとの関連性については，病理学的なデータが不足しており，今後の検討が必要である．

おわりに

現在の医学では不可逆的に進行した筋萎縮を治療することは不可能であるが，筋萎縮に至る前の早期段階で運動能力の可塑性(回復力)を科学的な根拠に基づく方法で診断することができれば，介護現場においてもさまざまな場面で有効に活用できるであろう．また，サルコペニアの原因を早期に発見することができれば，進行を抑制するための適切な処方の開発とその有効

性を検討することが可能となるであろう．

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3．運動神経細胞とサルコペニア

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- サルコペニア（加齢性筋肉減少症）は認知症と並んで，高齢者の activity of daily living（ADL）と quality of life（QOL）を損なう主要な原因である．
- サルコペニアの定義および診断に筋量，筋力と歩行速度が指標に用いられている．
- 早期発見，運動機能障害者に対するリハビリの効果の判定を可能にするためには，客観的かつ有効な新しいバイオマーカーの開発が必要である．
- 加齢による筋の老化促進の要因は，体内環境全体の変化，幹細胞とそれを維持する微少環境の老化，筋と運動神経細胞の相互作用維持システムの老化の3種類に分類することができる．
- 筋萎縮に至る前段階で，運動能力の可塑性（回復力）を科学的な根拠に基づく方法で定量化することができれば，介護現場においてもさまざまな場面で有効に活用できるであろう．

Key Words　サルコペニア，バイオマーカー，神経筋シナプス，逆行性シグナル，運動神経細胞

サルコペニア（加齢性筋肉減少症）は高齢者の activity of daily living（ADL）と quality of life（QOL）を損なう主要な原因である．すでに超高齢社会を迎えている我が国だけでなく欧米においてもサルコペニアの問題が注目されている．サルコペニアの特徴は加齢による筋肉量低下と筋力低下だが[1]，実際に臨床や介護現場で有効に役立てることができる定義と診断基準のいずれについても確立されていないのが現状である．さらに，サルコペニアは遺伝因子と環境要因に加えて，エピジェネティックな変化など多様な老化促進因子が長時間重なって起きるため，その病態とメカニズムの解明が困難となっている．現在，認知症は多くの病型に分類することができるが，サルコペニアも多様な原因により筋萎縮へ収束する病態の集合群であり，まだ単一の概念として捉えているだけであると考えられる．加えて，高齢者が何らかの原因で急性期の運動機能障害を抱えてしまうと，リハビリによる機能回復の程度は個人差が大きく困難なケースも多くなる．明らかな原因疾患（悪性腫瘍，重篤な感染症，脳血管障害，認知症）による筋肉喪失（cachexia）とサルコペニアの違いの明確な定義はないが，cachexia のケースで基礎疾患が改善された後のリハビリによる回復力の違いは，サルコペニアの成因と関連する可能性がある．本稿では，特に筋と運動神経の相互維持作用とサルコペニアの成因メカニズムについて紹介する．

□ サルコペニアの定義

サルコペニアの定義はまだ定まっていないのが現状であると述べた．一方で2010年に世界に先駆けてサルコペニアの定義が Europa Consensus として発表された[2]．筋量と筋力，および歩行速度の3つを指標にして，それぞれを組み合わせることでサルコペニアを診断するとしている（図1）．これらの指標は Janssen らの疫学研究の結果を基にしているが，さまざまな原因によって生じるサルコペニアを早期に診断して予防するために真に有効かどうか，既に疑問視する考え方もあり，今後も継続して検証する必要がある．上記の3つの指標や日常生活を指標とした運動機能能力のスコアーによる判定は，すでに筋萎縮を伴うような顕著な筋力低下を検出することは容易であるが，早期発見と予防に対して必ずしも有効であるとはいえない．サルコペニアも認知症と同じく症

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状が顕著になったケースでは病態が進行している．もし，上記の指標とは独立したサルコペニアのバイオマーカーが開発され利用できるようになると，早期発見，運動機能障害者に対するリハビリの効果の判定を可能にする，客観的かつ有効な指標として介護予防対策に有用となると期待される．サルコペニアはすでに超高齢社会を迎えた日本だけでなく，欧米においても医学的かつ社会的にも急務の問題とされているようであるが，早期発見とリハビリの有効性を判定できる診断法（指標）は未解決の課題である．サルコペニアは，さまざまな原因により誘発される終末像と考えられるが，その原因についてはほとんどわかっていないのが実情である．

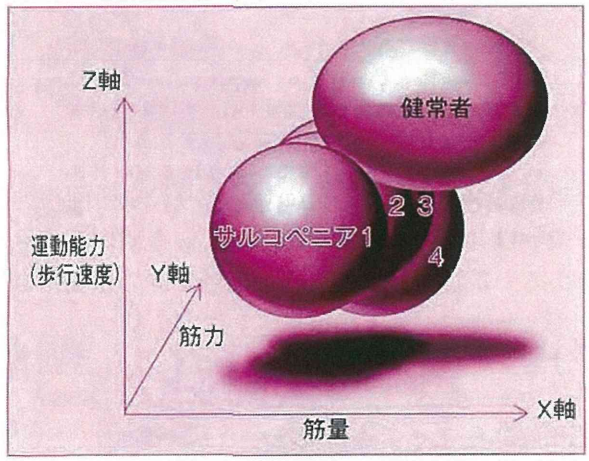

図1　サルコペニアの定義と診断

サルコペニアはさまざまな原因によって起きるが，それらを筋量，筋力および歩行速度の組み合わせで診断する意義については，今後の検討が必要である．

□ サルコペニアの3つの成因メカニズム

サルコペニアに関するこれまでの研究から，加齢による筋の老化促進の要因は以下の3種類に分類することができる（図2）．体内環境全体の変化（免疫・炎症，ホルモン，代謝・栄養状態），そして幹細胞（サテライト細胞）とそれを維持する微少環境（ニッチ）の老化，さらに筋と運動神経細胞（中枢神経）の相互作用による維持システムの老化である．これらの原因がお互いに影響しあうことでサルコペニアが進行すると考えられる．

筆者らは，運動神経線維と筋のつなぎ目である神経筋シナプスを介した筋と，運動神経の相互作用システムとサルコペニアとの関連に注目して研究を行っている．もともと健常筋には萎縮へと向かうカスケードが常在している．若い健常者であっても骨折などで筋活動が停止すると，2週間以内で急速に筋萎縮に至る．適切な運動習慣により，運動神経線維と筋のつなぎ目である神経筋シナプスを介した筋と，運動神経の相互作用システムが，萎縮カスケードに拮抗することで筋と運動神経の両方が保持されている[3,4]．筋と運動神経のシナプスを介した相互作用の分子機構は，未だよくわかっていない．特に筋から運動神経への逆行性シグナルの機能分子は，運動神経細胞に対する標的由来神経栄養因子として働いていると予想され，筋萎縮性疾患の病因解明と治療への応用が

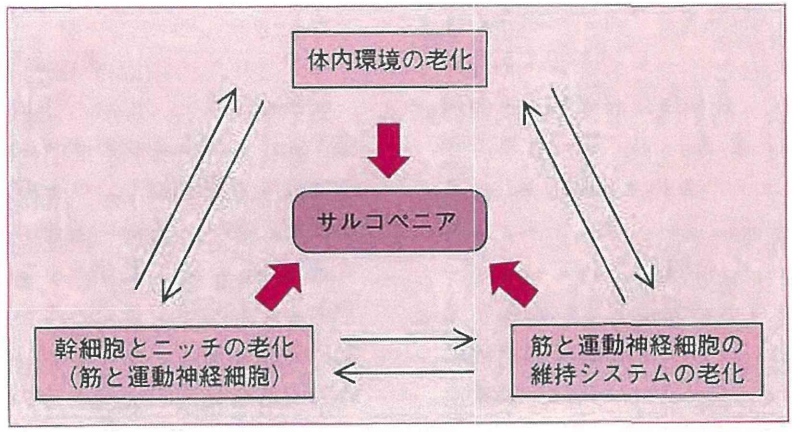

図2　サルコペニアのメカニズム

環境要因，遺伝因子，エピジェネティックの変化で誘発される3種類の経路

期待される．これまで同定されたNGFファミリーやGDNFなど，神経栄養因子の運動神経細胞に対して期待された臨床治療効果は限定的であるのが現状である．筆者らは，老化に伴う何らかの原因により，運動神経と筋の相互作用維持メカニズムが阻害されると筋萎縮が誘導されると考えている．このメカニズムを解明することで，サルコペニアの原因解明，診断と予防法の開発が可能となるかもしれない．

□ 加齢による筋の質的変化

サルコペニアでは筋萎縮に至るまでの過程で遅筋線維から速筋線維への質的変換が起き，そして神経筋シナプスを介して筋の質的変換を決定するとの考え方が20年以上も前に組織学的研究から示されている．遅筋はミトコンドリアによる代謝が主であり，一方で速筋は解糖系が優位であることから，老化による筋の質的変化は代謝変換も伴っていると考えられる．その分子メカニズムは未だ解明されていないが，筋の質的変化は運動神経細胞により誘導されると考えられている．速筋線維を支配するシナプスには選択的な脆弱性があり，近傍の遅筋線維を支配するシナプスから代償性に伸張した運動神経終末で再神経支配された時に速筋線維から遅筋線維へと転換するとしている．そして，再支配を受けることができなかった筋線維は萎縮する．一方で，筋の質的変化は運動神経細胞とは独立して，遺伝子転写補助因子であるperoxisome-proliferator-activated receptor-γ coactivator-1α（PGC1α）遺伝子をマウスに発現させると速筋が遅筋へ変換することが報告されている．運動神経との関係については不明であるが，筋が運動神経とは独立して質的変化を起こす可能性も予想される．PGC1αはIL-6，TNFαなどの炎症サイトカインの筋での発現を抑制する[5]．また，サルコペニアの原因についてはミトコンドリア機能との関連が古くから指摘されているが，PGC1αはミトコンドリアの機能を正に調節する（図3）．興味深いことにPGC1αを筋に強制的に発現させたトランスジェニックマウスは，高齢になっても筋の運動機能が保たれサルコペニアを抑制し，さらに寿命も顕著に長くなるという[5]．筋

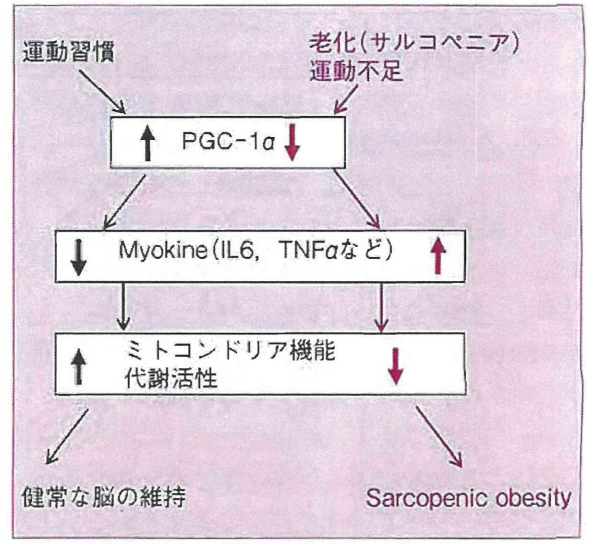

図3　サルコペニアとPGC-1αの関係を示す模式図

加齢とともに筋肉のPGC-1αの発現が減少して，筋から炎症性サイトカイン（myokine）の産生が増加する．また，筋のミトコンドリア機能が弱まり，萎縮した筋の代わりに脂肪組織が増える（sarcopenic obesity）．インシュリン感受性も減弱する．運動習慣はPGC-1αの発現を増強することで逆に作用する．

萎縮側索硬化症（ALS）に代表される運動ニューロン疾患でも臨床症状が出現する前から，筋萎縮に先立って速筋線維から遅筋線維への筋の質的転換が観察されることが指摘されている（図4）[6]．シナプスによる筋の質的変換と運動神経の選択的な脆弱性の分子メカニズムの解明は，筋と神経の相互作用と筋萎縮の分子病態を理解するうえで重要な鍵となると筆者らは考えている．

まとめ

現在の医学では不可逆的に進行した筋萎縮を治療することは不可能であるが，筋萎縮に至る前の早期段階で運動能力の可塑性（回復力）を科学的な根拠に基づく方法で定量化することができれば，介護現場においてもさまざまな場面で有効に活用できるであろう．また，サルコペニアの原因を早期に発見することができれば，進行を抑制するための適切な処方の開発とその有効性を検討することが可能となるであろう．

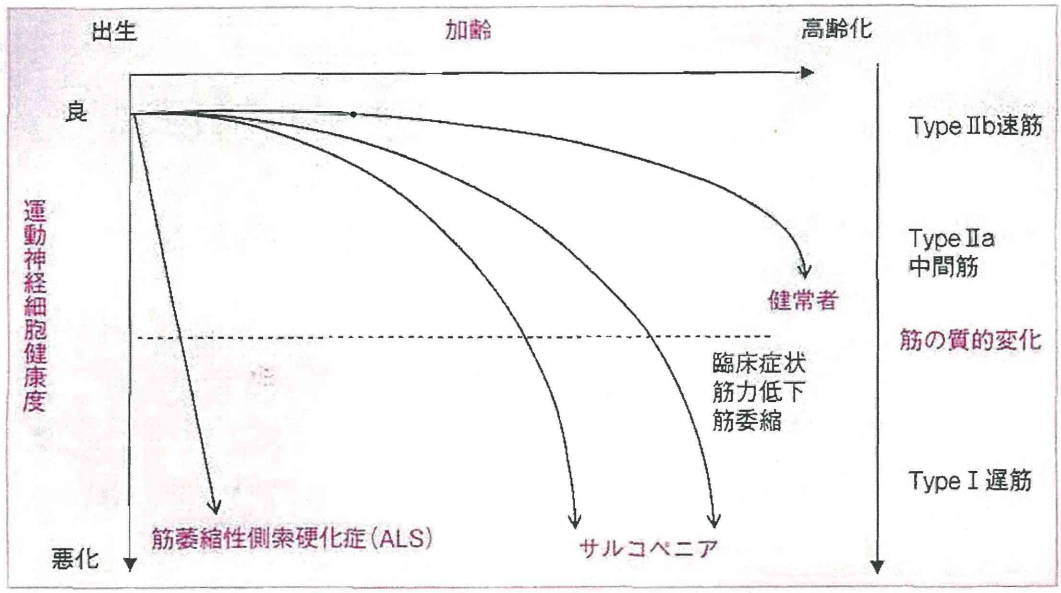

図4　サルコペニアと運動神経細胞の健康度の関係（仮説）

サルコペニアも筋萎縮側索硬化症も筋の質的変化（速筋の遅筋化）が起きることが報告されている．サルコペニアにおける運動神経細胞の消失との関係は未だ明らかではない．

（Frey D. et al. : J Neurosci 20 : 2534-2542. 2000 より引用して改変）[6]

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The effects of multidimensional exercise treatment on community-dwelling elderly Japanese women with stress, urge, and mixed urinary incontinence: A randomized controlled trial

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ABSTRACT

Background: Urinary incontinence is one of the most prevalent health problems and a significant cause of disability and dependence in the elderly. Pelvic floor exercise is effective in reducing stress urinary incontinence, but few studies have investigated the effect of behavioral management on urge and mixed incontinence.
Objectives: To determine the effects of multidimensional exercise treatment on reducing urine leakage in elderly Japanese women with stress, urge, and mixed urinary incontinence.
Design: Randomized controlled, follow-up trial.
Settings: Urban community-based study.
Participants: 127 community-dwelling women aged 70 and older with stress, urge, and mixed urinary incontinence were randomly assigned to the intervention ($n = 63$) or the control group ($n = 64$).
Methods: Urine leakage and fitness data were collected at baseline, and after the intervention and follow-up. The intervention group received a multidimensional exercise treatment twice a week for 3-month. After treatment, the participants were followed for 7-month.
Results: There were significant differences in changes of functional fitness and incontinence variables between the intervention and control groups. The intervention group showed urine leakage cure rates of 44.1% after treatment and 39.3% after follow-up ($\chi^2 = 21.96$, $p < 0.001$); whereas, the control group showed no significant improvement. The multidimensional exercise treatment was significantly effective in decreasing all three types of urinary incontinence. However, the effects of the exercise treatment were greater on stress urinary incontinence than on urge or mixed urinary incontinence. At the 7-month follow-up, while cure rates of all three types of urinary incontinence were significantly maintained, a slight reversal was seen only in the urge and mixed urinary incontinence ($\chi^2 = 10.28$, $p = 0.008$). According to the logistic regression model, urine leakage volume (adjusted odds ratio OR = 0.69, 95% confidence interval CI = 0.39–0.98), compliance (OR = 1.03, 95%CI = 1.01–1.16), and BMI reduction (OR = 0.67, 95%CI = 0.48–0.89) were significantly associated with the cure of urine leakage after intervention. The cure rate of urine leakage after the follow-up was significantly associated with compliance (OR = 1.13, 95%CI = 1.02–1.29) and BMI reduction (OR = 0.78, 95%CI = 0.60–0.96).
Conclusions: The intervention group showed higher urine leakage cure rates than control group. This result suggests that multidimensional exercise strategies may be effective for all three types of urinary incontinence. BMI reduction and compliance to the intervention was the consistent predictor for the effectiveness of the exercise treatment.

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What is already known about the topic?

- Several factors such as overweightness, high body mass index, and increased abdominal fat have been associated with higher risk of urinary incontinence.
- Behavioral management is effective in the treatment of stress urinary incontinence and is therefore recommended as a first-line therapy.
- Compliance has a positive influence on the effects of the exercise treatment.

What this paper adds

- Multidimensional exercise treatment targeting pelvic floor muscles and abdominal fat and/or BMI reduction are equally effective in reducing stress, urge, and mixed urinary incontinence after the intervention and follow-up.
- BMI reduction as well as compliance to the prescribed exercise regimen was the most consistently significant predictors of the short- and long-term effectiveness to the behavioral therapy.
- Multidimensional exercise treatment should be considered for the elderly as a strategy for reducing incontinence and improving functional capacity.

1. Introduction

Urinary incontinence (UI) in elderly people is a common condition that contributes greatly to the loss of independence, decrease in quality of life, restriction of social activities, and increase in risk for hospitalization or long-term care. The estimated prevalence of UI ranged from 17 to 55% depending on the definition of UI, the population characteristics, and the methodological approach (Thom, 1998). A number of methods are used to treat or deal with UI. Pelvic floor muscle (PFM) exercise, devised by Kegel (1948), is recommended as a first line of treatment in the management of stress UI and many investigators have validated the short- and long-term effects on stress UI (Cammu and Van Nylen, 1995; Goode et al., 2003; Kim et al., 2007). PFM exercise is hypothesized to enhance urethral resistance by increasing the strength and endurance of the periurethral and perivaginal muscles and by improving the anatomic support given to the bladder neck and proximal urethra (Kegel, 1951; Bo et al., 1999). One previous study found that PFM exercise reduces urine leakage in urge and mixed UI because of inhibition of the bladder reflex associated with PFM contraction; however, this study had no control group (Nygaard et al., 1996).

Several studies have reported that obesity and high BMI are associated with UI (Bump et al., 1992; Brown et al., 1999). One study reported objective and subjective resolution of stress and urge UI after surgically inducing weight loss in morbidly obese women (Bump et al., 1992). These results suggest that weight reduction is desirable for UI treatment (Subak et al., 2005; Auwad et al., 2008; Wing et al., 2010). We hypothesized that fitness exercises focused on strengthening the abdominal muscles would reduce abdominal fat and/or BMI, and thereby reduce abdominal wall pressure, intravesicular pressure, and the risk of UI in elderly women.

We conducted a randomized controlled trial to measure the effects of a multidimensional exercise treatment (FPM and fitness exercises) on urine leakage episodes in community-dwelling elderly Japanese women with stress, urge, and mixed UI, and to identify the factors that influence the effectiveness of the trial.

2. Methods

2.1. Subjects

The subjects in this study were randomly selected from the Basic Resident Register of 5935 women aged 70 and older that resided in the Itabashi ward (district) of Tokyo as of April 1, 2006. Information about the study was mailed to potential subjects. The baseline survey was conducted in November 2006, and 957 (16.1%) women participated. Out of the participants, 416 (43.5%) were experiencing some urinary incontinence, and 194 (46.6%) were classified as experiencing urine leakage more than once a week.

The inclusion criteria were: (1) suffering from urge, stress, or mixed UI; (2) being $\geq$70 years old; (3) having urine leakage episodes more than once a week; and (4) completing a 1-week urinary diary. The exclusion criteria included (1) an unclear UI type; (2) having urine leakage episodes less than once a week; (3) not completing the 1-week urinary diary; (4) impaired cognition (a Mini-Mental State Examination score of <24) (Folstein et al., 1975; McDowell et al., 1999) and (5) unstable cardiac conditions such as ventricular dysrhythmias, pulmonary edema, or other musculoskeletal conditions. Sixty seven (34.5%) of the potential participants were excluded because they were classified into one or more of the exclusion criteria. The study protocol was approved by the Clinical Research Ethics Committee of Tokyo Metropolitan Institute of Gerontology (TMIG). The procedures were fully explained to all participants, and written informed consent was obtained (Fig. 1).

2.2. Randomization

Randomization was performed after the baseline assessment and completion of the 1-week urinary diary, and any variable that identified personal information was not included in the randomization process. The assigned identification numbers of 127 participants (stress = 37, urge = 47, and mixed = 43) were divided into two groups based on the computer-generated random numbers. One group was randomly assigned to the intervention group (n = 63), and the other to the control group (n = 64). There was no attempt to equalize the size of the groups based on their characteristics or to recruit subjects with specific characteristics. The randomization procedure was blinded, and the investigators that evaluated the effects of the exercise treatment were blind to the allocation of interventions.

2.3. Outcome measures

The primary outcome of this trial was the cure rate of urine leakage episodes, which was assessed by the self-

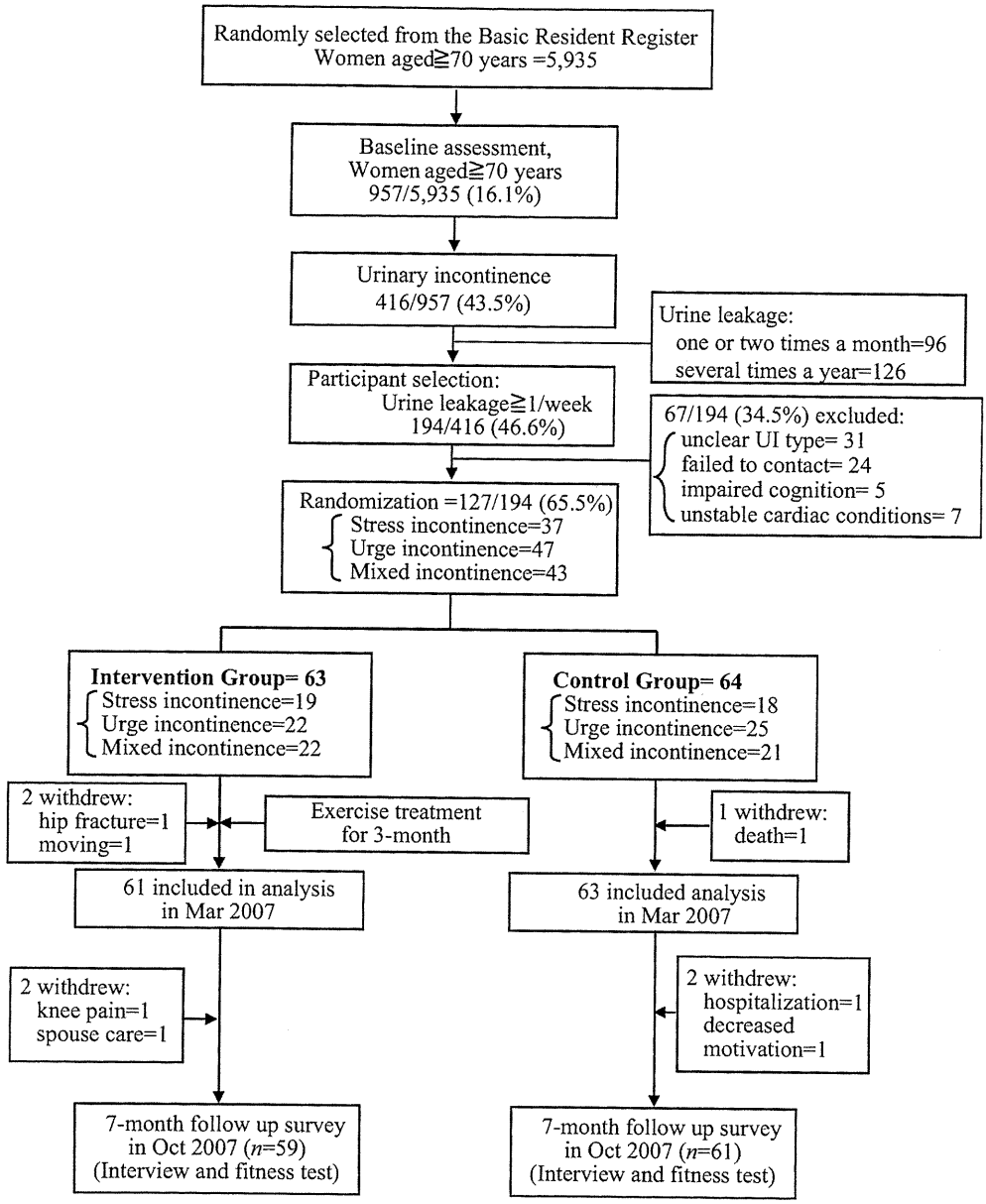

Fig. 1. Flow of the participants through the randomized controlled trial of exercise treatment and analyses.

reported urinary diary data (Wyman et al., 1988; Locher et al., 2001). The effects of the exercise treatment on urine leakage episodes were assessed based on the changes in the 5-point scale (1 = no urine leakage, 2 = less than once a week, 3 = once a week, 4 = two or three times a week, and 5 = everyday) recorded in the 1-week urinary diary, which was obtained pretreatment, after the 3-month exercise and 7-month follow-up. Complete cessation of urine leakage episodes was defined as cured.

2.3.1. Interview survey and urinary diary

A face-to face interview was conducted to assess temporary UI conditions based on the modified International Consultation on Incontinence Questionnaire (ICIQ) (Avery et al., 2004). The ICIQ was easily completed with low levels of missing data (mean 1.6%), good construct validity, and acceptable convergent validity. Reliability was good with moderate to very good stability in test-retest analysis and a Cronbach's alpha of 0.95 (Avery et al., 2004; Gotoh et al., 2009). The first question was "Have you experienced urine leakage during the previous year?" If the person responded that urine leakage episodes occurred more than once a week, to confirm the pretreatment frequency of urine leakage episodes, potential subjects were provided with a 1-week urinary diary. The subjects documented the time of every void and urine leakage episode, as well as the amount and circumstance of each episode. To confirm the changes of frequency, the urine leakage scores were calculated based on the self-reported 1-week urinary diary as follows: 0 = no urine leakage, 1 = less than once a week, 2 = once a week, 3 = two or three times a week, and 4 = every day.

UI type was classified based on inquiries about urine leakage in relation to 8 possible antecedents (Avery et al.,

2004). Stress UI was recorded when urine leakage was associated with increased abdominal pressure such as coughing, sneezing, or participating in some other physical activity. Urge UI was recorded when urine leakage was reported to be associated with running water or an urge to void and not being able to reach the toilet in time. When the characteristics of both stress and urge UI types were present, it was defined as mixed UI.

The subjects were asked about the onset age and duration of UI, frequency of daytime and nighttime voiding, and chronic medical conditions.

2.3.2. Functional fitness test

Measurements of height and body weight were converted to body mass index (BMI, kg/m^2), waist circumference, and fitness test variables including grip strength, usual and maximum walking speed, and seated hip adductor muscle strength. The procedures for the fitness tests have been described in detail in a previous report (Kim et al., 2007).

2.4. Intervention

2.4.1. Intervention group

The participants attended an exercise treatment session 2 times a week for 3-months at the TMIG health promotion classes. The protocols of the PFM and fitness exercises have been published previously (Kim et al., 2007). The following exercises were performed by the participants.

2.4.2. Stretching exercise

Before the PFM exercise and muscle strengthening training, the participants performed 5–10 min of warm-up and stretching exercises, including shoulder rotation, waist rotation, and others.

2.4.3. PFM exercises

The subjects were trained to exert force only on the PFM without excessively straining the abdomen. The exercise regimen was designed to strengthen the fast- and slow-twitch muscle fibers located on the pelvic floor. The participants were initially instructed to perform 10 fast contractions (3 s) with a 5-s relaxation and 10 sustained contractions (8–10 s) with a 10-s relaxation between the contractions. The PFM exercise was performed in the sitting, lying, and standing positions with the legs apart, while emphasizing contraction of the PFM and relaxation of the other muscles.

2.4.4. Fitness exercises

Strength training of the thigh and abdominal muscles was performed between the PFM exercises. The fitness exercise included: lifting the foot (or both feet) and pointing toes then slowly pulling toes back toward the shin, slowly lifting one knee (or both knees), tilting the pelvis backward and forward, lifting the buttocks while on the back with the knees bent, raising one leg while lying on the back, and others. The ball exercises included actions like sitting on the ball, rolling the ball and the pelvis forward and backward, and moving from side to side, squeezing the thighs, lifting the ball with the legs, and others.

2.4.5. Control group

The control group received general education classes once a month for three months, where participants were educated on cognitive function, osteoporosis, and oral hygiene.

2.5. Follow-up

During the 7-month follow-up period, the participants attended 1-h exercise classes once a month at the TMIG health promotion center. The home-based program consisted of two to three sets of the 13 exercises and the PFM exercises that they had learned during the group exercise sessions. They were encouraged to perform the home-based exercises at least three times per week for approximately 30 min per day. To accurately monitor the exercise times and the number of sets performed during the follow-up period, a pamphlet illustrating the PFM and strengthening exercises and a recording sheet were distributed to the subjects. The subjects were asked to document the time and sets of exercises performed at home each day, the urine leakage episodes, and the amount and circumstances of each episode. The record sheets were collected once a month at the group exercise class and analyzed to calculate the mean exercise frequency per week and the mean exercise time per day. When a participant was absent from an exercise class, we mailed the record sheet to the individual.

2.6. Data analysis

The sample size was calculated to allow detection of a 20.0% reduction in urine leakage episodes between groups with 80% power and a significance level of 0.05 (Burgio et al., 2002).

The mean differences between groups were analyzed using the *t*-test for continuous variables and the chi-square test for categorical variables. To evaluate the differences between the groups in the effects of the intervention on selected continuous variables and urine leakage score at the baseline, after the 3-month exercise, and at the 7-month follow-up, a repeated-measures two-way analysis of variance (ANOVA) was performed. Significant interactions were analyzed to determine whether the effects were greater in the intervention or control group. A repeated-measures one-way ANOVA was also performed to evaluate the within group. A *post hoc* analysis was performed using the Scheffe method. The generalized estimating equation was used to compare the effects between the groups after 3-month exercise and at the 7-month follow-up on the cure rate of UI. The Kruskal–Wallis test was used to evaluate the differences of UI type in the effect of the exercise treatment on urine leakage episodes. The Cochran Q-test was used to evaluate the within-group differences in the effect of the exercise program on urine leakage episodes for baseline, 3-month exercise, and follow-up data.

Multiple logistic regressions were performed to identify variables that were associated with cured urine leakage after 3-month of exercise and at the 7-month follow-up after intervention. All analyses were performed using SPSS software, Windows version 15.0 (SPSS, Inc., Tokyo, Japan).

Table 1
Selected variables characteristics of participants at baseline by study group.

Variables[a]	Intervention group ($n = 63$)	Control group ($n = 64$)	p Value[b]
Age (yr)	76.1 ± 4.3	75.7 ± 4.4	0.625
Height (cm)	148.4 ± 5.8	148.9 ± 6.2	0.639
Body weight (kg)	51.8 ± 8.7	54.0 ± 7.9	0.202
BMI (kg/m^2)	23.4 ± 3.3	24.3 ± 3.0	0.195
Waist circumference (cm)	78.9 ± 10.2	78.5 ± 9.9	0.853
Grip strength (kg)	19.2 ± 4.6	18.6 ± 4.7	0.561
Adductor muscle strength (kg)	20.6 ± 6.9	21.5 ± 4.8	0.502
Usual walking speed (m/s)	1.2 ± 0.3	1.1 ± 0.3	0.282
Maximal walking speed (m/s)	1.7 ± 0.4	1.7 ± 0.4	0.423
Onset age of incontinence (yr)	71.3 ± 7.6	71.0 ± 7.1	0.865
Period of incontinence (year)	4.8 ± 6.4	4.6 ± 6.0	0.890
Frequency of toilet in daytime (times)	7.7 ± 3.1	7.4 ± 2.3	0.525
Frequency of toilet in night (times)	1.9 ± 1.2	1.8 ± 1.3	0.581
Frequence of urine leakage (%)			
Everyday	46.0	50.0	0.714
1 every two days	11.1	7.8	
More than once a week	42.9	42.2	
Amount of urine leakage, large (%)	23.8	32.8	0.210
Chronic medical conditions, yes (%)			
Hypertension	57.1	57.8	0.918
Hyperlipemia	36.5	40.6	0.712
Diabetes	17.5	15.6	0.780

[a] Data are presented as M (mean) and SD (standard deviation) for continuous variables, and percentage for categorical variables. BMI = body mass index.
[b] Two group t-tests for continuous variables and chi-square test for categorical variables.

3. Results

3.1. Subjects characteristics and compliance

The baseline demographic, fitness, and interview variables of the participants in the two groups are summarized in Table 1. Most of the baseline characteristics were similar between the groups.

The attendance rate during the 3-month exercise treatment ranged from 63.5% to 81.1%, with a mean of 70.3%. Seven participants (intervention group = 4, control group = 3) were unable to complete the study after

Table 2
Comparison of functional fitness and incontinence variables between intervention ($n = 59$) and control ($n = 61$) after 3-months of exercises and the 7-month follow-up.

Variables[a]	G[b]	Baseline	3-month exercise	7-month follow-up	ANOVA[c] G × T	p Value
Body Weight (kg)	I	52.0 ± 8.9	51.9 ± 8.8	50.9 ± 8.9	$F = 5.78$	0.018
	C	53.9 ± 8.2	53.9 ± 8.2	53.9 ± 8.1		
BMI (kg/m^2)	I	23.7 ± 3.4	23.5 ± 3.0	23.2 ± 3.1	$F = 11.49$	0.001
	C	24.1 ± 2.9	24.0 ± 2.7	24.4 ± 3.4		
WC (cm)	I	78.8 ± 10.3	77.8 ± 9.7	77.7 ± 9.9	$F = 4.06$	0.041
	C	79.3 ± 10.4	79.2 ± 10.5	78.9 ± 9.6		
UWS (m/s)	I	1.2 ± 0.2	1.2 ± 0.2	1.2 ± 0.2	$F = 2.79$	0.099
	C	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.2		
MWS(m/s)	I	1.7 ± 0.4	1.8 ± 0.4	1.8 ± 0.4	$F = 5.10$	0.027
	C	1.7 ± 0.4	1.6 ± 0.3	1.6 ± 0.4		
GS (kg)	I	19.0 ± 4.7	20.7 ± 5.0	19.8 ± 5.7	$F = 0.37$	0.547
	C	19.0 ± 4.2	20.2 ± 3.5	19.5 ± 3.8		
AMS (kg)	I	20.5 ± 7.1	24.1 ± 7.7	24.3 ± 7.9	$F = 11.00$	0.001
	C	21.2 ± 4.8	22.1 ± 4.8	21.8 ± 4.9		
ULS (point)	I	5.0 ± 1.0	3.0 ± 2.0	3.6 ± 2.2	$F = 7.64$	0.007
	C	5.1 ± 1.0	4.4 ± 1.6	4.8 ± 1.6		
Cure of urine leakage	I	0.0	44.1	39.3	21.96	<0.001
	C	0.0	1.6	1.6		
Cure of urine leakage in intervention group	Stress	0.0	63.2[d]	66.7[e]	15.77	<0.001
	Urge	0.0	35.0[d]	26.1[e]	7.49	0.032
	Mixed	0.0	40.1[d]	30.0[e]	9.56	0.016

[a] Data are presented as mean and standard deviation.. WC = waist circumference; UWS = usual walking speed; MWS = maximum walking speed; GS = Grip strength; AMS = adductor muscle strength; ULS = urine leakage score.
[b] G = group, I = intervention group, C = control group.
[c] ANOVA = analysis of variance, T = time. Chi-square and p values are from generalized estimating equation. Conhran's Q-value.
[d] Kruskal–Wallis test: chi-square = 1.99, $p = 0.391$.
[e] Kruskal–Wallis test: chi-square = 10.28, $p = 0.008$. (Scheffe's *post hoc* = stress > urge, mixed urinary incontinence).