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特集　高齢者の薬物療法ガイドライン

Seminar＜疾患・症候別に考える＞

8．筋骨格領域(骨粗鬆症，関節リウマチを中心として)の薬物療法

石井　伸弥

KEY WORD　■骨粗鬆症　■骨折予防　■関節リウマチ　■病態修飾性抗リウマチ薬(DMARDs)　■非ステロイド性消炎鎮痛薬(NSAIDs)　■上部消化管出血

SUMMARY

■骨粗鬆症：①ビスフォスフォネート薬，テリパラチドの骨折予防効果は高齢者においても若年者と同等である．②選択的エストロゲン受容体モジュレーターは浸潤性乳がんを予防する．③デノスマブはビスフォスフォネート薬と同等の骨折予防効果を有する．④ビタミンD単独では骨折予防効果は弱いと考えられるが，カルシウムとビタミンDの組み合わせでは骨折予防効果をもたらす可能性がある．⑤カルシウム補充はビタミンDと併用しているか否かにかかわらず心血管系のイベント，特に心筋梗塞のリスクを増す可能性がある．

■関節リウマチ：①病態修飾性抗リウマチ薬は，高齢者において感染を含めた副作用の危険性が若年者より高まるが，関節リウマチをコントロールする効果は同様である．②非ステロイド性消炎鎮痛薬によって，特に高齢者において上部消化管出血の危険性が高まる．③非ステロイド性消炎鎮痛薬による上部消化管出血の危険性はMisoprostol，H_2ブロッカー，選択的COX-2阻害薬，プロトンポンプ阻害薬を用いて下げることができる．

はじめに

骨密度は年齢が高くなるにつれ低下するため，骨粗鬆症は年齢に伴って有病率が増加する．また閉経前後に大きく骨密度が低下することもあり，女性に多い疾患である．関節リウマチは50歳代前後の発症が多く，女性に多くみられる．発症年齢のピークは骨粗鬆症と比べるとやや低いものの，長い経過をたどる疾患であり，高齢の患者も多い．したがって，骨粗鬆症，関節リウマチのいずれもが高齢女性に比較的多くみられる疾患である．これらの疾患は慢性的な経過をたどるため，治療も長期にわたる．その一方で治療には多くの薬剤が用いられ，治療も複雑である．そのため，筋骨格系疾患の中でも特にこの2疾患を対象として，薬物療法上の留意点について述べる．

骨疾患(骨粗鬆症)

薬物療法(表1，2)

原発性骨粗鬆症の薬物治療開始基準は『骨粗鬆症の予防と治療ガイドライン2011年版』において，骨粗鬆症の診断基準を満たした場合またはFRAX®と呼ばれる骨折リスク評価ツールにおいて，10年間主要骨粗鬆症性骨折確率15％を上回った場合とされている．現在，ビスフォスフォネート薬，副甲状腺ホルモン薬(テリパラチド)，カルシトニン薬，ビタミンK薬，ビタミンD薬，選択的エストロゲン受容体モ

■いしい　しんや(東京大学大学院医学系研究科加齢医学)

表1　ストップ：中止を考慮するべき薬剤もしくは使用法のリスト

薬剤(クラスまたは一般名)	代表的な商品名	対象となる患者群	主な副作用・理由	推奨される使用法	参考にしたガイドラインまたは文献
非COX選択的非ステロイド性抗炎症薬(1日325 mg以上のアスピリン，サリチル酸，メフェナム酸，ジクロフェナク，スリンダク，モフェゾラク，エドトラク，ナブメトン，イブプロフェン，ロキソプロフェン，ナプロキセン，ピロキシカム，アンピロキシカム，メロキシカム，ロルノキシカムなど)	アスピリン®，バファリン®，ポンタール®，ボルタレン®，ブルフェン®，ナイキサン®，ロキソニン®，モービック®など	すべての高齢者	75歳以上，ステロイド使用，抗血小板薬，抗凝固薬などの危険因子をもつ患者群において胃腸出血，消化性潰瘍の危険性を高める．プロトンポンプ阻害薬またはミソプロストロール(サイトテック®)の使用は危険性を減らすが取り除くことはできない．消化性潰瘍，胃腸出血，消化管穿孔の危険性は3～6カ月の使用で1%，1年の使用で2～4%であり，長期使用に伴って上昇する．	可能な限り長期的な使用を避ける．長期的な使用が避けられない場合にはプロトンポンプ阻害薬またはミソプロストロール(サイトテック®)の使用を考慮する．消化性潰瘍の既往がある場合には使用を避ける．	2012 Beers Criteria[15] 2009 AGS Pain Guideline[16] 2008 STOPP and START[14]
インドメタシン	インダシン®，インテバン®など	すべての高齢者	上記非COX選択的非ステロイド性抗炎症薬と同様の副作用をもつが，最も副作用を起こす危険性が高い．	使用すべきではない	2012 Beers Criteria Table 2[15] 2009 AGS Pain Guideline[16]
上記非COX選択的非ステロイド性抗炎症薬(インドメタシン含む)に加えCOX-2選択的阻害薬(セレコキシブ)	セレコックス®	うっ血性心不全	水分貯留，うっ血性心不全の悪化の危険性がある．	使用すべきではない	2012 Beers Criteria[15] 2008 STOPP and START[14]
		推算糸球体濾過量30 mL/min以下の慢性腎不全	腎障害の危険性がある．	使用すべきではない	2012 Beers Criteria[15] 2008 STOPP and START[14]
		高血圧	高血圧悪化の危険性がある．	使用すべきではない	2008 STOPP and START[14]
		ワルファリン(ワーファリン®など)使用	消化管出血の危険性がある．	使用すべきではない	2008 STOPP and START[14]

表2　スタート：強く推奨される薬剤もしくは使用法のリスト

薬剤（クラスまたは一般名）	代表的な商品名	推奨される使用法	注意事項	参考にしたガイドラインまたは文献
DMARD	リウマトレックス®，ブレディニン®など	活動性の関節リウマチの診断がついた場合	薬剤の選択は関節リウマチの活動状態や個々の患者の全身状態による 高齢者では薬物有害事象や易感染性の危険性が高まるため，治療開始前および開始後定期的にモニタリングを行う	2008 STOPP and START[14] 2013 EULAR recommendations for the management of rheumatoid arthritis[11] 2012 Update of the 2008 ACR recommendations for the use of DMARDs and biologic agents in the treatment of rheumatoid arthritis[10] (Diaz-Borion, 2009)[12] (Bathon, 2006)[13]
ビスフォスフォネート	ボナロン®，アクトネル®など	骨粗鬆症の診断基準を満たしたとき，または骨粗鬆症性脆弱骨折の危険性が高いと判断された場合	骨折リスクおよび患者の全身状態に応じてテリパラチド，選択的エストロゲン受容体調節薬，デノスマブを用いてもよい	2008 STOPP and START[14] 2011 骨粗鬆症の予防と治療ガイドライン[17] (Hochberg, 2005)[1] (Bone, 1997)[2] (Miller, 2005)[3] (Boonen, 2010)[4] (Marcus, 2003)[5] (Vogel, 2008)[6] (Lin, 2012)[7]

ジュレーター（Selective Estrogen Receptor Modulators：SERM），カルシウム薬，デノスマブなどが骨粗鬆症に対して用いられる代表的な薬剤である．このうち，ビスフォスフォネート薬，テリパラチドは高齢者においても骨折予防効果は若年者と同等であるという結果が，比較対照試験のサブ解析より得られている[1-5]．またSERMは，浸潤性乳がんの予防効果があることがメタ解析によって示されており[6]，乳がん高リスク患者に対してよい適応があると考えられる．デノスマブは，ビスフォスフォネート薬と同等の骨折予防効果があることがメタ解析により示されている[7]．高齢者の骨粗鬆症に対しての第一選択は，使用経験や安全性のデータが最も得られているビスフォスフォネート薬であるが，ビスフォスフォネート薬はその煩雑な服用法や消化器症状がアドヒアランス低下の一因となることがあり，また重篤な腎機能低下においては投与量の調節が必要である．したがって，全身状態なども考慮した上でテリパラチドやSERM，デノスマブもよい選択肢であると考えられる．

ビタミンK薬やビタミンD薬の骨折予防効果は，ほかの薬剤と比べて強くない．カルシウム薬と組み合わせることで，ビタミンDは骨折予防効果をもたらす可能性はあるが[8]，カルシウム薬は心血管系のイベント，特に心筋梗塞のリスクを増すことがメタ解析によって示されており，使用に当たっては心血管系リスクの評価を含め慎重であるべきである[9]．

筋疾患（関節リウマチ）

薬物療法（表1，2）

関節リウマチに対し，アメリカリウマチ学会（American College of Rheumatology）と欧州リ

ウマチ学会(European League Against Rheumatism)いずれもが診断がつき次第，早期からの病態修飾性抗リウマチ薬(Disease Modifying Anti-Rheumatic Drugs：DMARDs)による薬物治療開始を推奨している[10,11]．これは，早期からDMARDsによって関節リウマチの疾患活動性を低下させることで関節破壊の進行を防止し，機能維持を目指すことを目的としている．DMARDsは大きく分けて，低分子化合物と生物学的製剤に分けられる．初回治療での薬剤の選択は関節リウマチの活動度や全身状態によって決まるが，メトトレキサートが用いられることが多い．高齢患者においては，易感染性を含め副作用の発現する危険性が高い[12]．だがその一方で，いくつかのDMARDsにおいては高齢患者に対しても若年患者と同様に優れた効果を発揮することが，比較対照試験のサブ解析によって示されている[12,13]．そのため，高齢であることを理由としてDMARDsによる治療から除外されるべきではないが，DMARDsの使用に当たっては治療開始前の感染症スクリーニングおよび開始後の有害事象モニタリングをより慎重に行うべきである．DMARDsは効果の発現までに数カ月を要するため，その期間関節症状を速やかに軽減するステロイドが併用されることもある．しかし，ステロイドの種々の副作用(骨粗鬆症，感染症，消化管潰瘍，脂質・血糖値上昇，白内障など)を考慮して，その期間は6カ月程度にとどめ，その後は減量されるべきである[10]．ステロイド単剤治療は推奨されない[10,14]．また，痛みや腫れなどの症状に対して，非ステロイド性抗炎症薬が対症療法として用いられる．しかし，高齢者においては副作用の危険性が高く，可能な限り短期的な使用にとどめるべきである．胃腸出血，消化管穿孔の危険性は，プロトンポンプ阻害薬やヒスタミンH_2受容体拮抗薬によって軽減できることが知られている[14-16]．

文　献

1) Hochberg MC et al；FIT Research Group：Effect of alendronate on the age-specific incidence of symptomatic osteoporotic fractures. J Bone Miner Res 2005；**20**(6)：971-976.
2) Bone HG et al：Dose-response relationships for alendronate treatment in osteoporotic elderly women. Alendronate Elderly Osteoporosis Study Centers. J Clin Endocrinol Metab 1997；**82**(1)：265-274.
3) Miller PD et al：Safety and efficacy of risedronate in patients with age-related reduced renal function as estimated by the Cockcroft and Gault method：a pooled analysis of nine clinical trials. J Bone Miner Res 2005；**20**(12)：2105-2115.
4) Boonen S et al：Assessment of the relationship between age and the effect of risedronate treatment in women with postmenopausal osteoporosis：a pooled analysis of four studies. J Am Geriatr Soc 2010；**58**(4)：658-663.
5) Marcus R et al：The skeletal response to teriparatide is largely independent of age, initial bone mineral density, and prevalent vertebral fractures in postmenopausal women with osteoporosis. J Bone Miner Res 2003；**18**(1)：18-23.
6) Vogel VG：Managing the risk of invasive breast cancer in women at risk for breast cancer and osteoporosis：the role of raloxifene. Clin Interv Aging 2008；**3**(4)：601-609.
7) Lin T et al：Comparison of clinical efficacy and safety between denosumab and alendronate in postmenopausal women with osteoporosis：a meta-analysis. Int J Clin Pract 2012；**66**(4)：399-408.
8) Avenell A et al：Vitamin D and vitamin D analogues for preventing fractures associated with involutional and post-menopausal osteoporosis. Cochrane Database Syst Rev 2009；(2)：CD000227.
9) Bolland MJ et al：Calcium supplements with or without vitamin D and risk of cardiovascular events：reanalysis of the Women's Health Initiative limited access dataset and meta-analysis. BMJ 2011；**342**：d2040.
10) Smolen JS et al：EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs：2013 update. Ann Rheum Dis 2014；**73**(3)：492-509.
11) Singh JA et al：2012 update of the 2008 American College of Rheumatology recommendations for the use of disease-modifying antirheumatic drugs and biologic agents in the treatment of rheumatoid arthritis. Arthritis

Care Res(Hoboken) 2012；64(5)：625-639.
12) Díaz-Borjón A：Guidelines for the use of conventional and newer disease-modifying antirheumatic drugs in elderly patients with rheumatoid arthritis. Drugs Aging 2009；26(4)：273-293.
13) Bathon JM et al：Safety and efficacy of etanercept treatment in elderly subjects with rheumatoid arthritis. J Rheumatol 2006；33(2)：234-243.
14) Gallagher P et al：STOPP (Screening Tool of Older Person's Prescriptions) and START (Screening Tool to Alert doctors to Right Treatment). Consensus validation. Int J Clin Pharmacol Ther 2008；46(2)：72-83.
15) American Geriatrics Society 2012 Beers Criteria Update Expert Panel：American Geriatrics Society updated Beers Criteria for potentially inappropriate medication use in older adults. J Am Geriatr Soc 2012；60(4)：616-631.
16) American Geriatrics Society Panel on Pharmacological Management of Persistent Pain in Older Persons：Pharmacological management of persistent pain in older persons. J Am Geriatr Soc 2009；57(8)：1331-1346.
17) 骨粗鬆症の予防と治療ガイドライン作成委員会(日本骨粗鬆症学会，日本骨代謝学会，骨粗鬆症財団)編：骨粗鬆症の予防と治療ガイドライン2011年版，ライフサイエンス出版，東京，2011.

(執筆者連絡先) 石井伸弥 〒113-8655 東京都文京区本郷7-3-1 東京大学大学院医学系研究科加齢医学

救急医に必要な高齢者医療の最新の知識；診かた，評価法（身体面）

A review of current knowledge of geriatric medicine for emergency physicians：Evaluation of physical findings

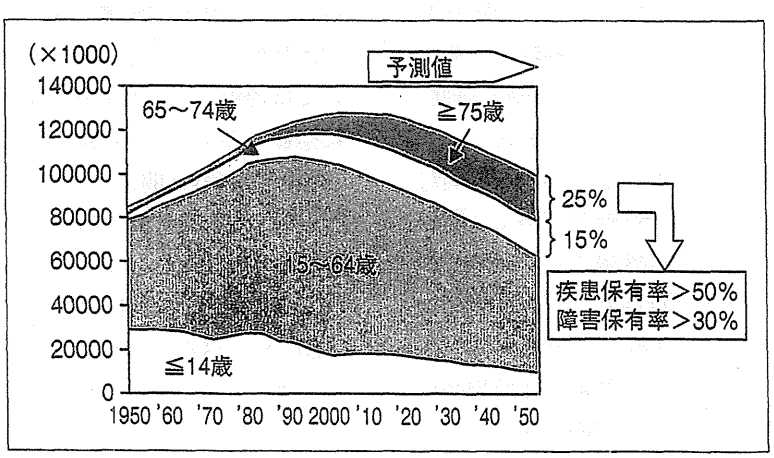
石井　伸弥*　　秋下　雅弘*
Shinya Ishii　　*Masahiro Akishita*

◆key words：多病，老年症候群，高齢者総合的機能評価，高齢者に対する適切な医療提供の指針

はじめに

65歳以上の高齢者が総人口に占める割合，いわゆる高齢化率はついに25％を超え[1]，明確な定義はないものの，超高齢社会に突入したと考えてよいであろう。しかし，超高齢社会の抱える真の問題は高齢化率ではなく，75歳以上の後期高齢者の増加にある。図1に示すように，前期高齢者の人口はほぼ横ばい，さらには減少へ転じる一方で，後期高齢者は増え続け，2050年には4人に1人が後期高齢者という時代になると予測されている。その後期高齢者の半数は大きな慢性疾患を有し，30％は要介護状態にあるという状況は，今後20～30年の医学・医療の進歩があってもさほど改善しないと見込まれており，医療機関の多くが後期高齢者で占められることは避けられない。厚生労働省の患者調査によると，すでに全国の外来患者の23％，入院患者の46％は後期高齢者である。消防庁の平成25年現況調査では救急自動車による搬送人員における高齢者の割合は53.1％である[3]。事故種別ごとにみていくと，交通事故を除いて急病，一般負傷，その他のいずれの分類においても高齢者がもっとも高い割合を占めていた。この現況調査では後期高齢者の割合は示されていないが，後期高齢者がかなりの割合を占めていると考えられる。

このように，われわれが今後直面する高齢者医療は，元気に通院する前期高齢者ではなく，多くの慢性疾患に加え高齢者特有の症状（老年症候群），日常生活障害を抱え，しばしば救急搬送される後期高齢者を主な対象としたものになると考えられる。そのため，臓器横断的で生活環境にも配慮した包括的な医療が必要である。救急診療におけるアセスメントは主訴に重点をおいたものになりがちであるが，



〔文献2）より引用・改変〕

後期高齢者の著増がみられる

図1　日本における年齢別人口構成の推移

*　東京大学医学部附属病院老年病科

表1　高齢者の疾患・病態上の特徴

1. 複数の疾患を有する
2. 老年症候群が増加する
3. 認知機能など日常生活障害を抱える
4. 症状が非定型的である
5. 薬物に対する反応性が異なる
6. 社会的因子の影響が大きい

救急外来においても患者の日常生活機能，認知機能，気分・意欲を系統的に評価する高齢者総合的機能評価は予後を改善し得ることが示されている[4]。時間がかかる高齢者総合的機能評価を救急受診した全高齢患者に行うことは現実的ではないが，虚弱で多疾患をもつハイリスク患者を対象として高齢者総合的機能評価の中から焦点を絞った必要な評価を行い，みつけ出された問題点に対して適切な医療機関・専門科を紹介することは可能であると考えられる。

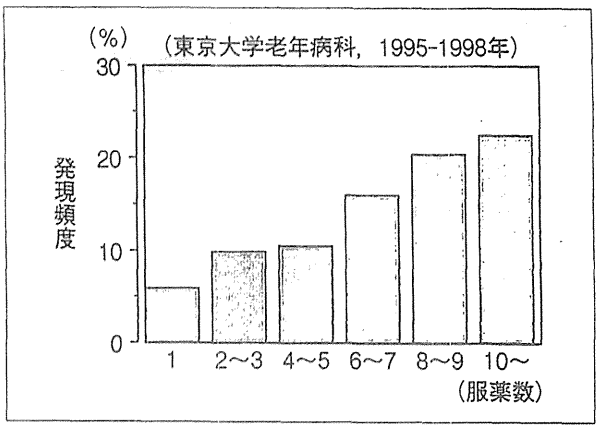
本稿では，まず高齢患者の特徴をまとめ，それに関連して高齢者総合的機能評価について言及する。次にその背景となる身体機能の加齢変化を概説する。身体所見をとるうえでの注意事項もここで述べる。

高齢患者の特徴

高齢者の疾患・病態上の特徴を**表1**にまとめたが，とくに後期高齢者によく当てはまる。前期高齢者にも慢性疾患は多くみられるものの，比較的元気で日常生活機能も保たれている患者が多い。後期高齢者にも元気な患者はいるが，複数の慢性疾患と老年症候群を有し，日常生活に障害を抱え，介護を要する患者の比率が多くなる。ただ，高齢者では個人差が非常に大きくなり，暦年齢だけでは単純に判断することができない点には注意が必要である。

まず複数の疾患（多病）であるが，高齢者の疾患は生活習慣病をはじめとする慢性疾患が多く，臓器の老化が基盤にあるため治癒は困難であるという特徴がある。またこれに伴って慢性的に処方されている薬剤数が増え，多剤服用（polypharmacy）となりやすい。**図2**に示すように，多剤併用は服用や処方の過誤，薬剤の相互作用によって薬物有害作用を引き起こす危険性が高い。多剤服用の患者においては薬物有害作用が起こっている可能性を念頭において評価を行うことが重要である。

多病に加え，高齢者は老年症候群もまた複数もっていることがある。老年症候群とは，高齢者に特有



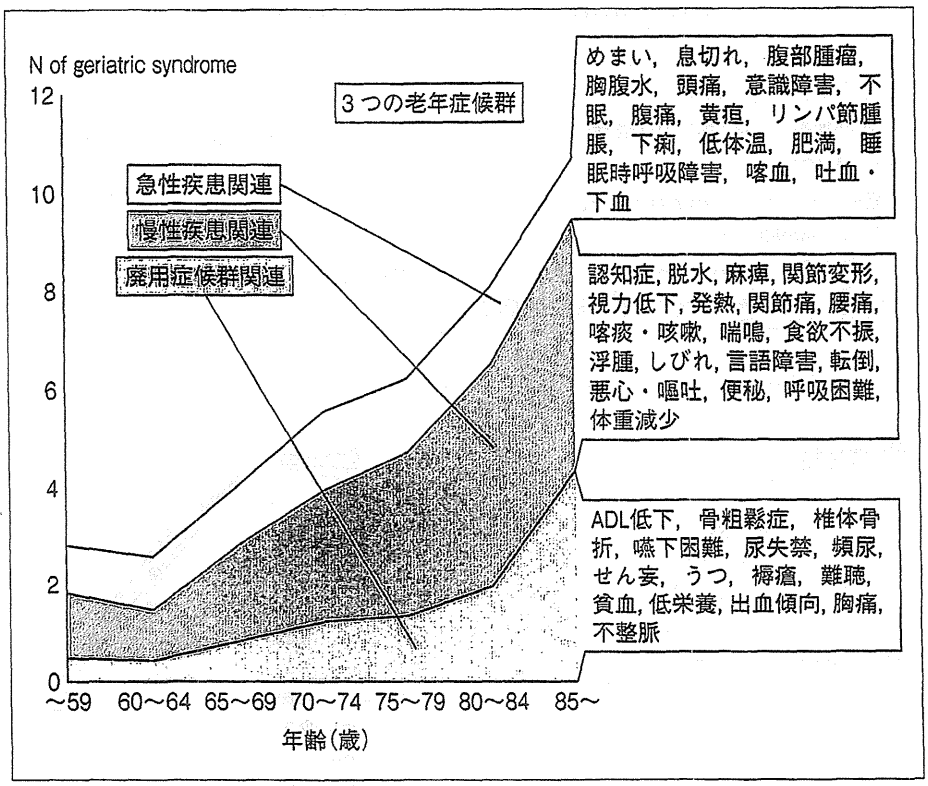
〔文献5)より引用・改変〕

図2　服薬数と薬物有害作用発現頻度

もしくは高頻度に認める症候で，包括的な対処を要するものと定義される。一般に原因は多彩かつ多岐にわたり，さまざまな臓器にまたがるため，有効な治療法は少なく，QOLの低下をもたらし障害要因にもなることが特徴である。認知症，尿失禁，難聴など非常に頻度の高いものから，嚥下困難，転倒など頻度はやや低くても，肺炎や骨折といった重大な問題につながるものまでさまざまな症候がある。実際には多くの高齢患者が複数の症候を抱えている。**図3**に示すように年齢が高くなるにつれて老年症候群の数も増加する。このような多様かつしばしば複数併存する老年症候群であるが，時間軸によって分類すると理解しやすい。それぞれ急性疾患に関連するもの，慢性疾患に関連するもの，廃用症候群に関連するものである。急性疾患関連のものは年齢が高くなっても比較的一定の頻度で起こっているが，慢性疾患や廃用症候群に関連するものは年齢が高くなるにつれて急激に頻度が高まる。これらの老年症候群は月単位，年単位で生じるものも多く，診療においては治療によって可逆的な病態であるかどうか常に念頭におくことが必要である。

疾患や症候と関連して日常生活に支障をきたすことも高齢患者の特徴である。日常生活機能にはさまざまな側面があり，基本的日常生活動作（activities of daily living；ADL）から手段的さらには社会的ADLとレベルが上がり，それぞれに評価尺度がある。**表2**に基本的ADLの指標であるBarthel indexの項目を示す[8]。移動とセルフケアに分けられるが，これらに障害があると日常的に介護が必要になる。**表3**には手段的ADLの指標として代表的なLawtonによる評価尺度の各項目を示すが，独居機能を評価するのに有用である[9]。

症状が非定型的である点については，胸痛のない



〔文献6)より引用・改変〕

図3　老年症候群と年齢

表2　基本的ADL（日常生活動作）

移動
移乗，移動，階段昇降，食事，入浴
セルフケア
トイレ動作，排尿コントロール，排便コントロール，更衣，整容，食事，入浴

〔文献8)より引用・改変〕

表3　手段的ADL（Lawton & Brody）

- 電話の使用
- 乗り物の利用
- 家計管理
- 買い物
- 服薬管理
- 食事の準備
- 掃除などの家事
- 洗濯

〔文献9)より引用・改変〕

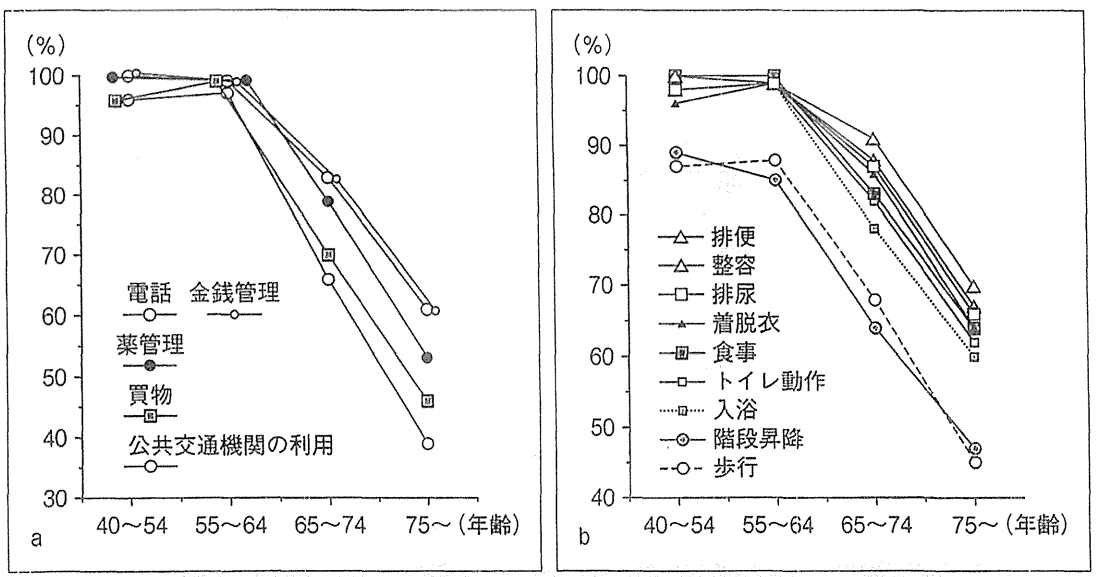
心筋梗塞，呼吸器症状のない肺炎，腹痛のないイレウスなど，若年成人では通常認められる症候を欠く病態が診断を難しくする。低血糖や肺炎が意識障害で初めて発見されるなど，結果的に重篤化と治療の遅れにつながる点が大きな問題である。食事，発語などの基本的な行動に変容をきたす，急に理解が悪くなったなどの変化は，背景に急性病態が隠れている可能性を考えて慎重に対処することが肝要である。また，認知機能の低下により訴えがはっきりしない，視力の低下により皮膚の変化に気づかないことなどもあり，訴えがないからといって症状や症候がないとは限らない点にも注意を要する。

社会的因子とは，生活環境や経済状況などを指し，高齢者では独居のため家族による日常的な介護を受けられないことや年金では生活できない，あるいは施設に入れないといった現象が問題となる。近年では，高齢者や認知症患者が互いに介護する老老世帯や認認世帯も社会問題化している。社会的因子は医療で解決できるものではなく，医療提供体制を含めて地域や多職種との連携が重要である。

これらを総合的に評価する手法が高齢者総合機能評価（comprehensive geriatric assessment；CGA）とよばれるもので，表4に示すような評価ツールを用いて手術適応や術後の管理，退院後の療

表4　高齢者総合機能評価の主な構成要素と標準的スケール

1. 基本的 ADL：Barthel index
2. 手段的 ADL：Lawton
3. 認知機能：改訂長谷川式知能評価スケール（HDS-R），mini-mental state examination（MMSE）
4. 気分：geriatric depression scale（GDS）
5. 意欲：vitality index
6. 問題行動：dementia behavior disturbance（DBD）Scale
7. 療養・生活環境：家族構成，要介護認定など



a：手段的 ADL（Lawton の各項目），b：基本的 ADL（Barthel index の各項目）

図4　男性患者における ADL 各項目自立度の加齢変化（東京大学医学部附属病院老年病科入院症例）

養環境の判断にも利用される。さまざまな算定要件はあるが，入院中にスクリーニングを含めた CGA を実施すれば総合評価加算100点の診療報酬が付けられるため，導入する病院が急増している。CGA のスクリーニングおよび評価ツールの詳細については，日本老年医学会のホームページに掲載されている内容[10]，およびそこから電子版にリンクできる『健康長寿診療ハンドブック』[7]の巻末資料を参考にしていただきたい。

身体機能の加齢変化

筋・骨格機能と脳神経機能の加齢変化，および心身機能に影響する諸臓器の加齢変化と疾患が加わる結果，加齢に伴い身体機能は低下する。前述した手段的 ADL と基本的 ADL について，男性入院症例における各項目の自立度と年齢の関係を図4に示すが，65歳以上，さらに75歳以上になるといずれの項目も自立の割合が低下する。筋・骨格機能の低下は女性のほうで顕著な結果が出ており，女性は男性に比べて移動能力を中心とした基本的 ADL が低下しやすい。筋量・筋力の低下は，上肢より下肢，とくに大腿四頭筋など大腿伸筋群で顕著である。このような部位差は生活上の筋活動に由来するとされるが，高齢者で問題となるのが移動能力の低下など主に下肢の障害であることを考えると，予防やリハビリテーションに際して考慮に入れるべき点である。

また，加齢に伴って生じる身体面での変化のため，身体所見の解釈が若年者と異なってくる点にも注意が必要である。ここでは例として高齢者の脱水に対する身体所見の有用性を**表5**に示す。これらの身体所見においてある程度の有用性が認められたのは腋窩の乾燥，口腔粘膜の乾燥，溝状舌のみであったが，それらの有用性も限られていた[11)12)]。皮膚の緊張感（ツルゴール）は高齢者においては信頼性が低い。起立性低血圧も高齢者においては脱水の有用な所見ではない[13)]。このように，一般的に高齢者においては身体所見の感度，特異度ともに若年者と比べると低いことが多く，有用性も限られていることが多い。したがって，診断に際しては1つの所見だけに頼る

表5　高齢者の脱水に対する身体所見有用性

所見	感度	特異度
腋窩の乾燥	50%	82%
口腔粘膜の乾燥	85%	58%
溝状舌	85%	58%
上眼瞼の凹み	62%	82%
混乱	57%	73%
脱力	43%	82%
発語の異常	56%	82%

のではなく，病歴や身体所見，検査所見などを総合的に判断するアプローチが必要である。

おわりに

以上のように，高齢者の心身機能は低下し，それ自体が疾患につながるだけでなく，老年症候群や日常生活機能障害として主疾患およびその治療にも大きな影響を与える。こうした高齢患者の特徴を理解して医療を行うことを目的として，考慮すべき事柄を「高齢者に対する適切な医療提供の指針」として厚生労働省の研究班でまとめた。表6に到達目標を示すが，全文は指針を共同作成した日本老年医学会，全国老人保健施設協会，日本慢性期医療協会のホームページに掲載されているので参照いただきたい[14]。個々の疾患診療ガイドラインが高齢患者を対象としていない場合，またはガイドラインが相互に矛盾する内容を含む場合などには，本指針に示された基本的な考え方を準用して治療方針決定の一助とすることができる。また，本指針は高齢者用の診療指針作成や施策立案の際にも参考になると思われる。

表6　高齢者に対する適切な医療提供の指針

医療従事者が高齢患者に対して医療提供を行う際に考慮すべき事柄を整理し，基本的な要件を示したもの
(到達目標のみ抜粋)

- 高齢者の病態と生活機能，生活環境をすべて把握する
- 生活機能の保持，症状緩和などによりQOLの維持・向上を目指す
- 患者のQOL維持に生活の場の問題は重要であり，適切な医療提供の場を選択する
- 医療提供の場を変更する際に生じる問題を理解し，予防に努める
- 有害作用や服薬管理，優先順位に配慮した薬物療法を理解し，実践する
- 意思決定支援の重要性を理解し，医療提供の方針に関して合意形成に努める
- 家族をはじめとした介護者の負担を理解し，早期に適切な介入を行う
- 患者もチームの一員であることを理解し，患者本人の視点に立った多職種協働によるチーム医療を行う

〔文献14)より引用・改変〕

【文　献】

1) 総務省統計局：人口推計. http://www.stat.go.jp/data/jinsui/pdf/201406.pdf (accessed 2014-07-01)
2) 内閣府ホームページ：平成23年版高齢社会白書(全体版). http://www8.cao.go.jp/kourei/whitepaper/w-2011/zenbun/23index.html (accessed 2014-07-03)
3) 総務省消防庁：救急救助の現況. http://www.fdma.go.jp/neuter/topics/kyukyukyujo_genkyo/h25/01_kyukyu.pdf (accessed 2014-07-01)
4) Graf CE, Zekry D, Giannelli S, et al：Efficiency and applicability of comprehensive geriatric assessment in the emergency department：A systematic review. Aging Clin Exp Res 23：244-254, 2011.
5) 鳥羽研二，秋下雅弘，水野有三，他：薬剤起因性疾患．日老医誌 36：181-185, 1999.
6) 日本老年医学会編：老年医学系統講義テキスト，西村書店，東京，2013.
7) 日本老年医学会編：健康長寿診療ハンドブック；実地医家のための老年医学のエッセンス，メジカルビュー社，東京，2011.
8) Mahoney FI, Barthel DW：Functional evaluation：The Barthel Index. Md State Med J 14：61-65, 1965.
9) Lawton MP, Brody EM：Assessment of older people：Self-maintaining and instrumental activities of daily living. Gerontologist 9：179-186, 1969.
10) 日本老年医学会ホームページ. http://www.jpn-geriat-soc.or.jp/kensyu/siryou.html (accessed 2014-07-03)
11) Gross CR, Lindquist RD, Woolley AC, et al：Clinical indicators of dehydration severity in elderly patients. J Emerg Med 10：267-274, 1992.
12) Eaton D, Bannister P, Mulley GP, et al：Axillary sweating in clinical assessment of dehydration in ill elderly patients. BMJ 308：1271, 1994.
13) McGee S, Abernethy WB 3rd, Simel DL：The rational clinical examination：Is this patient hypovolemic? JAMA 281：1022-1029, 1999.
14) 日本老年医学会ホームページ：高齢者に対する適切な医療提供の指針. http://www.jpn-geriat-soc.or.jp/proposal/pdf/geriatric_care_GL.pdf (accessed 2014-07-03)

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Coffee treatment prevents the progression of sarcopenia in aged mice in vivo and in vitro

Yinting Guo [a,b], Kaijun Niu [a,*], Tatsuma Okazaki [c], Hongmei Wu [a], Takeo Yoshikawa [d], Takashi Ohrui [e], Katsutoshi Furukawa [f], Masakazu Ichinose [c], Kazuhiko Yanai [d], Hiroyuki Arai [f], Guowei Huang [a], Ryoichi Nagatomi [g]

[a] Department of Nutrition and Food Science, School of Public Health, Tianjin Medical University, Tianjin, China
[b] Department of Behavioral Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan
[c] Department of Respiratory Medicine, Graduate School of Medicine, Tohoku University, Sendai, Japan
[d] Department of Pharmacology, Graduate School of Medicine, Tohoku University, Sendai, Japan
[e] Division of Geriatric Pharmacotherapy, Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan
[f] Department of Geriatrics and Gerontology, Division of Brain Sciences, Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan
[g] Division of Biomedical Engineering for Health & Welfare, Tohoku University Graduate School of Biomedical Engineering, Sendai, Japan

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ABSTRACT

Sarcopenia is characterized by the age-related loss of muscle mass and strength, which results in higher mortality in aged people. One of the mechanisms of the sarcopenia is the loss in the function and number of muscle satellite cells. Chronic low-grade inflammation plays a central role in the pathogenesis of age-related sarcopenia. Accumulating evidence suggests that coffee, one of the most widely consumed beverages in the world, has potential pharmacological benefits such as anti-inflammatory and anti-oxidant effects. Since these effects may improve sarcopenia and the functions of satellite cells, we examined the effects of coffee on the skeletal muscles in an animal model using aged mice. In vivo, coffee treatment attenuated the decrease in the muscle weight and grip strength, increased the regenerating capacity of injured muscles, and decreased the serum pro-inflammatory mediator levels compared to controls. In vitro, using satellite cells isolated from aged mice, coffee treatment increased the cell proliferation rate, augmented the cell cycle, and increased the activation level of Akt intracellular signaling pathway compared to controls. These findings suggest that the coffee treatment had a beneficial effect on age-related sarcopenia.

1. Introduction

The population of aged people over 60 years old is currently growing at the rate of 2.6% per year, which is more than twice the rate of the total population in the world (United Nations, 2009). In general, aging is accompanied by frailty, functional limitations, and disabilities that interfere with the activities of daily life. These factors reduce the quality of life of aged people and eventually cause their loss of autonomy in daily life. Sarcopenia is the age-related loss of the muscle mass and strength, which causes frailty, functional limitations in daily living, disabilities, and finally, a higher mortality rate in aged people (Altun et al., 2012).

Satellite cells are resident myogenic progenitors in the skeletal muscles. They play a central role in the growth and regeneration of the skeletal muscles (Hawke and Garry, 2001). In response to stimulation, satellite cells form myoblasts, fuse together and generate new fibers (Clemmons, 2009). The age-related functional disability and decrease in the number of satellite cells contribute to the development of sarcopenia (Welle, 2002). Thus, maintaining the functions of satellite cells and their numbers might reduce sarcopenia and, furthermore, might improve the regenerating capacity of the skeletal muscles in aged people.

Chronic low-grade inflammation plays a central role in the pathogenesis of age-related sarcopenia (Beyer et al., 2012). With aging, the levels of serum pro-inflammatory mediators such as tumor necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6) increase, and are inversely related to muscle mass, muscle strength, and disability in aged people (Cohen et al., 1997; Ferrucci et al., 1999; Greiwe et al., 2001; Visser et al., 2002). TNF-α decreases the expression levels of MyoD messenger RNA, a well-established skeletal muscle-specific transcription factor that directly regulates the expression of myogenic proteins and resulting in muscle wasting (Cai et al., 2004; Guttridge et al., 2000). IL-6 induces skeletal muscle atrophy in mice (Haddad et al., 2005; Tsujinaka et al., 1996).

Abbreviations: TNF-α, tumor necrosis factor-alpha; IL-6, interleukin 6; TA, tibialis anterior; eMyHC, embryonic myosin heavy chain.

* Corresponding author at: Department of Nutrition and Food Science, School of Public Health, Tianjin Medical University, 22 Qixiangtai Road, Heping District, 300070 Tianjin, China.
E-mail address: nkj0809@gmail.com (K. Niu).

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Coffee is one of the most widely consumed beverages in the world, and it has been considered to have beneficial capacities to one's health. Accumulating evidence suggests that coffee intake has numerous potential pharmacological benefits, such as antioxidant (Sato et al., 2011), antitumor (Franco, 2008), anti-diabetic (Ong et al., 2012), and anti-inflammatory effects (Chang et al., 2010; Shen et al., 2010).

Since the above effects of coffee might have the potential to improve sarcopenia and the functions of satellite cells (Macaluso and De Vito, 2004; Meng and Yu, 2010; Schaap et al., 2006; Sriram et al., 2011; Strasser et al., 2007), we hypothesized that coffee intake might have a beneficial effect on the prevention of sarcopenia. Furthermore, we hypothesized that this effect might involve inflammation. To the best of our knowledge, few studies have examined the effect of coffee intake on muscles in aged people or aged animals, or the relationship between coffee intake and pro-inflammatory mediator levels. Thus, in the present study, we examined the effects of coffee treatment on muscle weight, muscle strength, satellite cell functions, regenerating capacity of the skeletal muscles in vivo and in vitro, and the involvement of pro-inflammatory mediators in an animal model using aged mice.

2. Material and methods

2.1. Mice and drink treatment

Male C57BL/6 mice were obtained from Clea Japan (Tokyo, Japan) and maintained under specific pathogen-free conditions with unrestricted access to food and water. Experiments were carried out in accordance with the guidelines established by the Tohoku University Committee on Animal Research. At the age of 27-months, mice were divided into 2 groups according to the beverages provided for each group and maintained for the next 4 weeks, with 12 mice in each group. The 2 groups were: normal water (controls) and coffee. Ready-to-drink coffee (Clear®) was purchased from Nescafe (Sizuoka, Japan) and diluted with distilled water at 1:2 dilutions. In this study, all of the coffee products were from the same lot number. Japan Food Research Laboratories (Tokyo, Japan) analyzed the main components of the coffee. The coffee used in this study contained: coffee bean, ≈2.5 g/100 ml; chlorogenic acid, 0.07 mg/ml; anhydrous caffeine, 0.45 mg/ml; polyphenols, 1.4 mg/ml; specific gravity, 1.005 (at 20 °C). The water and coffee were changed every day. The mice had unrestricted access to food and water. Two mice in the control and one mouse in the treatment group died of natural causes during the treatment period. After 4 weeks of treatment, 4 mice from the control group and 5 mice from the coffee group were anesthetized, sacrificed, their sera were collected, and the weights of the hind limb muscles were measured. The hind limb muscles included the tibialis anterior muscle (TA), triceps surae muscle, quadriceps muscle, biceps femoris muscle, gluteus maximus muscle, and iliopsoas muscle. For evaluation of the myogenic progenitor proliferation and differentiation and fusion capacity of injured skeletal muscles, mice were sacrificed 3 or 5 days after the injury, respectively, 3 mice in each group.

2.2. Isolation and culture conditions of satellite cells and cell proliferation assay

Satellite cells were isolated from untreated, 28-month-old mice according to our previous study (Niu et al., 2013). Briefly, the hind limb muscles including the TA, triceps surae muscle, quadriceps muscle, biceps femoris muscle, gluteus maximus muscle, and iliopsoas muscle were isolated, non-muscle tissues were removed, and then the muscles were subjected to enzymatic dissociation with 0.2% collagenase type II (Worthington Biochemical Corporation, Lakewood, NJ, USA) for 60 min, then with 0.04 U/ml dispase (Gibco BRL, Grand Island, NY, USA) for 45 min. The cell suspension was filtered through a cell strainer (BD Bioscience, Franklin Lakes, NJ, USA), incubated with anti-mouse CD16/CD32 monoclonal antibody (mAb, 2.4G2, BD Bioscience) to block Fc receptors, then with the following antibodies: FITC-labeled anti-CD31, anti-CD45 (BD Bioscience), anti-CD11b, and anti-Sca-1 antibodies (eBioscience, San Diego, CA, USA); PE-labeled anti-Integrin α7 (MBL, Nagoya, Japan); Alexa 647-labeled anti-CD34 (BD Bioscience). The cells were sorted by a FACSAria™ II flow cytometer (BD Bioscience) as previously described (Niu et al., 2013).

Sorted satellite cells were cultured in growth medium containing high-glucose Dulbecco's modified Eagle's medium (HG-DMEM) with 20% fetal bovine serum (FBS) (MP Biomedicals, Morgan Irvine, CA, USA), 2.5 ng/ml basic fibroblast growth factor (bFGF, Invitrogen, Eugene, OR, USA), 100 U/ml penicillin, and 100 μg/ml streptomycin (Sigma, St. Louis, MO, USA). Satellite cells under 8 passages were used in this study. Diluted coffee solution was sterilized by a filter then added to the culture medium at the following concentrations: 10, 30, 50, or 100 μg/ml. The cells were cultured for 72 h, and the number of cells was determined by a water-soluble tetrazolium-8 (WST-8, DOJINDO, Tokyo, Japan) assay using a cell counting kit (Okazaki et al., 2008).

2.3. Locomotor activity recordings

Freely moving locomotor activity was recorded by an infrared ray sensor system (SUPERMEX®; Muromachi-Kikai, Tokyo, Japan) that consisted of 12 small compartments divided by walls on a large shelf. Each compartment (width: 40 cm × depth: 50 cm × height: 35 cm) was equipped with a ceiling sensor that can detect heat energy radiated from a mouse. The system detected mouse movement by recording changes in heat energy in the covered field. Mice were individually placed in a plastic cage (width: 19 cm × depth: 27.5 cm × height: 17 cm) and then put into the system shelf. Counts were measured every 10 min (Inoue et al., 1996). Locomotor activity was consecutively measured on days 0–1, 5–6, 10–11, and 19–20.

2.4. Grip strength test

Grip strength was measured by an electronic grip strength meter (MK-380; Muromachi Kikai). Mice were put on the fence and pulled back slowly. The point at which mice released the fence was determined as the grip strength. The measurements were repeated 3 times and maximal readings were taken (Arai et al., 2001). The grip strength was measured twice a week.

2.5. Cell-cycle analysis by flow cytometry

DNA synthesis in cells was evaluated by measuring BrdU incorporation (BrdU Flow Kits; BD Biosciences, San Jose, CA, USA) by flow cytometry. Briefly, 5×10^5 cells were cultured overnight. Then, the cells were stimulated for the next 72 h with 10, 30, 50, or 100 μg/ml coffee bean extract sterilized with a 0.22 μm filter. The cells were labeled with BrdU during the final 2 h of stimulation. The cells were then permeabilized, fixed, and stained with an anti-BrdU antibody coupled with FITC according to the manufacturer's protocol. Flow cytometry data were collected using a logarithmic scale, and the percentage of BrdU-positive cells was determined (Niu et al., 2012).

2.6. Muscle injury model

After 4 weeks of the coffee treatment, the mice were anesthetized and cardiotoxin from Naja mossambica mossambica (Sigma) dissolved in 100 μl phosphate-buffered saline (PBS) (10 μM) was injected into the TA. Three or five days later, the mice were sacrificed, the TA muscles were isolated, frozen in 2-methylbutane precooled in liquid nitrogen, and stored at −80 °C for the following histological analysis (Uezumi et al., 2010).

2.7. Immunohistochemistry and immunocytochemistry

Frozen muscle tissues were sectioned from a region approximately 3 mm from the top of the TA (8 μm in thickness) using a cryostat. For embryonic myosin heavy chain (eMyHC) staining, frozen sections were fixed with acetone/methanol (50%/50%) for 30 s at −20 °C. Specimens were blocked with 1% BSA, 0.1% Triton X-100 in PBS at room temperature for 45 min, then incubated with anti-eMyHC antibody (F1.652, DSHB, Iowa City, IA, USA) at a 1:2 dilution at 4 °C overnight, followed by Rodamine conjugated-secondary antibody staining (Chemicon International, Temecula, CA, USA) at room temperature in the dark for 1 h. Finally, the sections were mounted in Vectashield Mounting Medium with 4′,6-diamidino-2-phenylindole (DAPI) (Vector labs, Burlingame, CA, USA). In vivo, the regenerating capacity of the injured skeletal muscles was evaluated by quantifying the percentage of eMyHc-immunoreactive area per field (Fukada et al., 2012). Ten randomly selected fields at ×200 magnification were measured in each sample. ImageJ software was used to quantify the eMyHC-immunoreactive areas per field. For ki-67 staining, after quenching endogenous peroxidase with 3% H_2O_2 in PBS for 15 min, the sections were incubated with primary antibodies at 4 °C overnight (anti-ki67 antibody, 1:40 dilution; DAKO, Tokyo, Japan), followed by incubation with biotinylated anti-rabbit immunoglobulin G antibody using Histofine (Max-PO (Multi), Nichirei Bioscience, Osaka, Japan) according to the manufacturer's instructions. Then, the antibody complex was visualized with 3,30-diaminobenzidine tetrahydrochloride (MERCK, Darmstadt, Germany) (Niu et al., 2012). Images were taken using a phase-contrast and fluorescence microscope BZ9000 (Keyence, Osaka, Japan) (Asada et al., 2009).

2.8. Western blot analysis

Akt and phosphorylated-Akt (phospho-Akt) proteins of the satellite cells were detected by western blot analysis. Some cells were serum-starved overnight, then stimulated with 10 nM insulin (Sigma), which is a potent activator of Akt, for 5 min as a positive control. Western blot analysis was performed with a SDS-PAGE Electrophoresis System as describe previously (Yamanda et al., 2009). In brief, the cells were rinsed twice with ice-cold PBS and lysed using RIPA Lysis Buffer (Upstate, Temecula, CA, USA). The extracted protein fraction was electrophoresed in a sodium dodecyl sulphate-10% polyacrylamide gel and then transferred onto an Immobilon transfer membrane (Millipore, Bedford, MA, USA). The amount of protein loaded onto the gels was 36 μg per well. The membranes were immunoblotted with the primary antibodies to Akt and phospho-Akt (Cell Signaling, Boston, MA, USA) at 1:1000 dilutions. Then the membranes were incubated with horseradish peroxidase-conjugated anti-rabbit immunoglobulin G (Cell Signaling) at 1:25,000 dilution and the protein bands were detected with an enhanced chemiluminescence detection kit (Amersham, Buckinghamshire, UK) (Yamanda et al., 2009).

2.9. Enzyme-Linked Immunosorbent Assay (ELISA)

Sera were isolated from the inferior vena cava of the mice (Okazaki et al., 2007). The serum levels of IL-1α, IL-1β, IL-6, TNF-α, and IGF-1 were measured using a specific ELISA kit (R&D Systems) according to the manufacturer's instructions, respectively (Okazaki et al., 2007). The minimum detectable levels were 2.5, 3.0, 1.6, 5.1, and 3.5 pg/ml for IL-1α, IL-1β, IL-6, TNF-α, and IGF-1, respectively.

2.10. Statistical analysis

To determine the sample size, a power analysis was performed based on the results of previously performed research (Niu et al., 2013) and a preliminary experiment. The mean hind limb muscle-weight divided by the body weight of 28-month-old mice was 0.009. We estimated ≥20% (standard deviation: 0.0005) difference between the control and coffee groups in the hind limb muscle-weight divided by the body weight. Assuming an alpha error of 0.05 with a power of 0.90, we calculated a necessary sample size of 3 to show a significant effect. Based on this calculation and to ensure reasonable data, we increased the sample size to 4 or 5 in this study. The same calculation was applied to determine the sample size of myogenic progenitor proliferation and regenerating capacity test in vivo. Based on previously performed research (Niu et al., 2013) and a preliminary experiment, we estimated ≥30% (mean Ki67-positive cell number per field in control group ≈ 40 cells, SD ≈ 3.5) and ≥70% (mean relative eMyHc positive area in control group ≈ 9%, SD ≈ 1.8) difference in the myogenic progenitor proliferation and regenerating capacity test, respectively, between the control and coffee treatment groups. Assuming an alpha error of 0.05 with a power of 0.90 we calculated a necessary sample size of 3 to show a significant effect both in the myogenic progenitor proliferation and regenerating capacity test.

Data were presented as mean ± standard error (SE). Differences were analyzed by one-way analysis of variance (ANOVA) test (Post hoc, Tukey). The Spearman correlation coefficient (r) was calculated to evaluate the relationship between two continuous variables. All the tests for statistical significance were 2 sided, and $p < 0.05$ was considered statistically significant. All in vitro experiments were repeated at least 3 times. In this study, the main experiments such as grip strength measurement, cardiotoxin injection, and histological quantifications were blindly carried out.

3. Results

3.1. Coffee-treated mice had greater muscle weight and grip strength than controls

To examine the effects of coffee treatment on aged mice in vivo, we divided 27-month-old mice into 2 groups and treated them with either normal water (controls) or coffee for 4 weeks. Two mice in the control and one mouse in the treatment group died of natural causes during the treatment period. These mice were excluded from the analysis. During the intervention period, the body weight changed similarly in the coffee-treated and control groups (Fig. 1A) (p value > 0.89). The amounts of daily diet intake and drink were not different between the groups (Fig. 1B and C). We also examined the effect of coffee on locomotor activity in the aged mice. Experiments were performed under light: dark cycles of 12 h:12 h. Locomotor activity was not different between the groups (Fig. 1D). A previous study compared hind limb muscles-weight divided by body weight between 2, 8, and 24 month old mice and showed progressive loss of hind limb muscles-weight / body weight with aging, suggesting the progression of sarcopenia with aging (Niu et al., 2013). The hind limb muscles included the TA, triceps surae, quadriceps, biceps femoris, gluteus maximus, and iliopsoas muscles. Coffee treatment significantly increased the hind limb muscle-weight compared to controls (Fig. 1E). Similarly, we measured the weight of the bilateral hind limb muscles and divided by the body weight. Coffee treatment increased the weight of the hind limb muscles per body weight compared to control by 13.1% (0.011 ± 0.0005 for the coffee treatment group vs 0.0098 ± 0.0007 for the control group, mean ± SE, Fig. 1F). To examine the effect of coffee on the muscle strength, we performed the grip strength test. Consistent with the effect of coffee on the muscle mass, the coffee group had greater grip strength than the controls, suggesting that coffee improved the grip strength (Fig. 1G). Furthermore, a comparison between before and after the treatment period within the controls showed that grip strength decreased after the treatment period compared to before, suggesting the progression of age-related atrophy in muscle function during this period. In contrast, no significant changes were observed within the coffee group during the treatment period. These data suggested that coffee

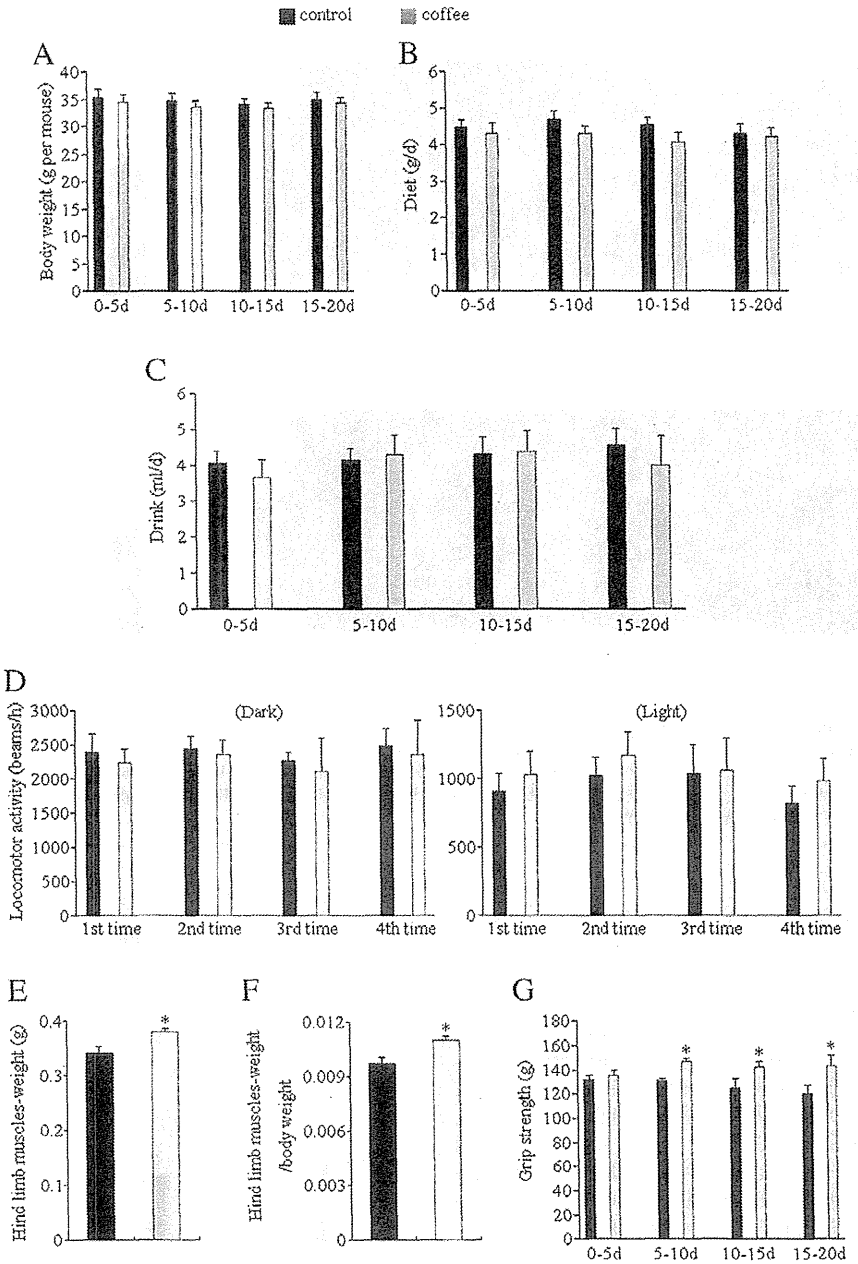

Fig. 1. Effects of coffee treatment on aged mice in vivo. Twenty-seven month-old mice were treated with coffee for 4 weeks. (A–C) Coffee treatment did not change body weight (A), the amount of the daily diet intake (B), or the volume of water intake (C). (D) Coffee did not change the daily locomotor activity levels. (E) The coffee-treated group had greater hind limb muscle-weight than the controls. (F) The coffee-treated group had greater hind limb muscles-weight per body weight than the controls. (G) The coffee-treated group had greater grip strength than controls. Columns are mean ± SE, n ≥ 4 in each group. *$p < 0.05$ compared with control.

treatment prevented the progression of atrophy in muscle weight and function in the aged mice.

3.2. Coffee treatment accelerated the regeneration of injured skeletal muscles

We next examined the effect of coffee treatment on the regenerating capacity of the skeletal muscles in aged mice in vivo by injuring the TA muscles with cardiotoxin injection and observed their regeneration. We isolated the muscles 3 or 5 days after the cardiotoxin injection. To determine the effect of coffee on the cell proliferation rate, we immunohistochemically stained muscle tissues for the cell proliferation marker Ki67 three days after the injury (Fig. 2A, left panels). The number of Ki67 immunoreactive cells was greater in the coffee group than that in controls, suggesting a greater cell proliferation rate in the coffee group (Fig. 2A, right panel). To confirm the regenerating capacity of the skeletal muscles, we immunohistochemically stained the muscle tissues for eMyHc, which is a marker of immature myotubes including regenerating muscles, 5 days after the injury (Fig. 2B, left panels). Quantification of the eMyHc immunoreactive area showed greater immunoreactive areas in the coffee group than in the controls (Fig. 2B, right panel). These results suggested that coffee treatment accelerated the regeneration of the injured skeletal muscles.

3.3. Coffee treatment decreased serum pro-inflammatory mediator levels

Since coffee has been suggested to have an anti-inflammatory effect, we examined the effect of coffee treatment on serum pro-inflammatory mediator levels in the aged mice. We chose IL-1α, IL-1β, IL-6, and TNF-α as pro-inflammatory mediators (Okazaki et al., 2003, 2009), and

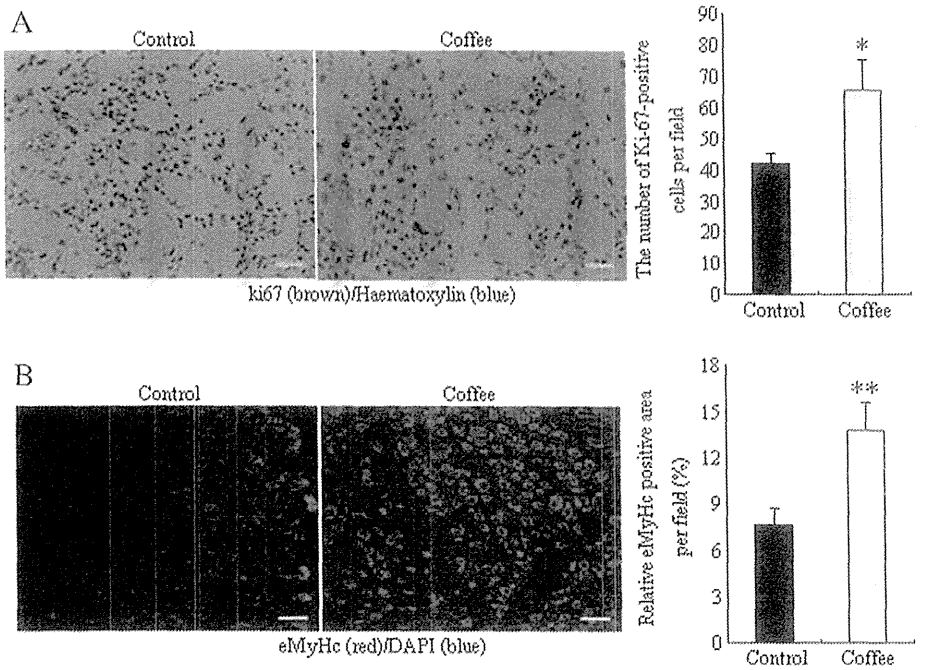

Fig. 2. Coffee treatment accelerated the regeneration of the injured skeletal muscles in aged mice. After 4 weeks of coffee treatment, we injected cardiotoxin into the tibialis anterior muscles of the mice to injure the muscles and isolated them 3 days later for (A) or 5 days later for (B). (A) The left panels are immunohistochemical staining for ki67 of the injured tibialis anterior muscles. The right graph is quantification of the number of ki-67 positive cells per visual microscopic field for each group (10 randomly selected fields at ×200 magnification per sample were quantified). (B) The left panels are immunohistochemical staining for eMyHC and DAPI of the injured tibialis anterior muscles. The right graph is quantification of the percentage of eMyHc-immunoreactive area per field for each group (10 randomly selected fields at ×200 magnification per sample). Scale bars, 100 μm. Columns are mean ± SE, n = 3 in each group. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with controls.

measured their levels in the serum. The levels of IL-1α, IL-6, and TNF-α were decreased in coffee treated group compared to controls (Fig. 3A). The correlation coefficients suggested a relationship between the serum pro-inflammatory mediator levels and the grip strength ($r = -0.38$, $r = -0.34$, $r = -0.42$, and $r = -0.36$ for IL-1α, IL-1β, IL-6, and TNF-α, respectively [$p < 0.05$ for all]). The correlation coefficients also suggested a significant relationship between several serum pro-inflammatory mediator levels and the muscle weight ($r = -0.52$, $r = -0.39$, $r = -0.69$, and $r = -0.63$ for IL-1α, IL-1β, IL-6, and TNF-α, respectively [$p < 0.05$ for IL-6 and TNF-α]). Since IGF-1 plays a central role in stimulating satellite cells, we also measured the serum levels of IGF-1. The serum levels of IGF-1 were not different (Fig. 3B).

3.4. Effects of coffee on the satellite cells of the aged mice in vitro

To examine the effect of coffee on the proliferation rate of the satellite cells of the aged mice in vitro, we isolated satellite cells from aged mice and stimulated them with coffee in growth medium for 72 h (Fig. 4A). Under the growing condition, coffee treatment increased the number of proliferating satellite cells compared to controls in a dose-dependent manner in vitro. Next, to examine the effect of coffee on the cell cycles of the satellite cells, we cultured the satellite cells for 72 h with coffee and measured DNA synthesis by BrdU incorporation using flow cytometry (Fig. 4B). The coffee-treated group had a greater BrdU incorporation rate than the controls (Fig. 4C). These results suggested that coffee enhanced the DNA synthesis of the proliferating satellite cells of the aged mice. The Akt signaling pathway plays a key role in the proliferation and cell cycle progression of the satellite cells (Giovannini et al., 2008; Kandarian and Jackman, 2006). Therefore, we next examined the activation level of Akt by western blot for Akt and the activated form of Akt, phosphorylated Akt. Coffee treatment increased the intensity of the bands of phosphorylated Akt compared to controls, which suggests that coffee treatment increased the activation

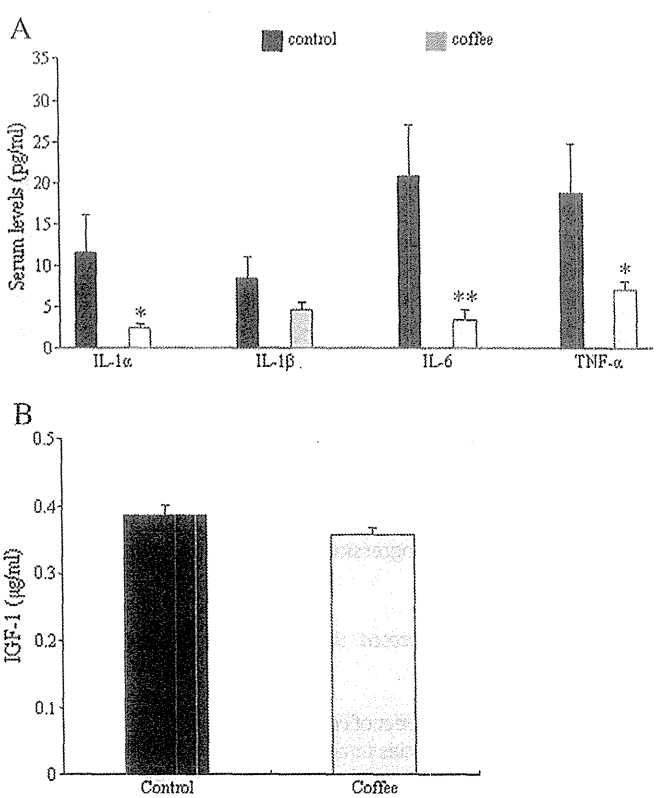

Fig. 3. Coffee treatment decreased the serum pro-inflammatory mediator levels, but did not affect the serum IGF-1 levels. ELISA determined serum levels of pro-inflammatory mediators and IGF-1 in aged mice treated with coffee for 4 weeks. (A) Coffee treatment significantly decreased the serum levels of IL-1α, IL-6, and TNF-α compared to controls. (B) Coffee treatment did not change the serum levels of the IGF-1 levels. Columns are mean ± SE, ≥4 in each group. $^{*}p < 0.05$, and $^{**}p < 0.01$ compared with control.

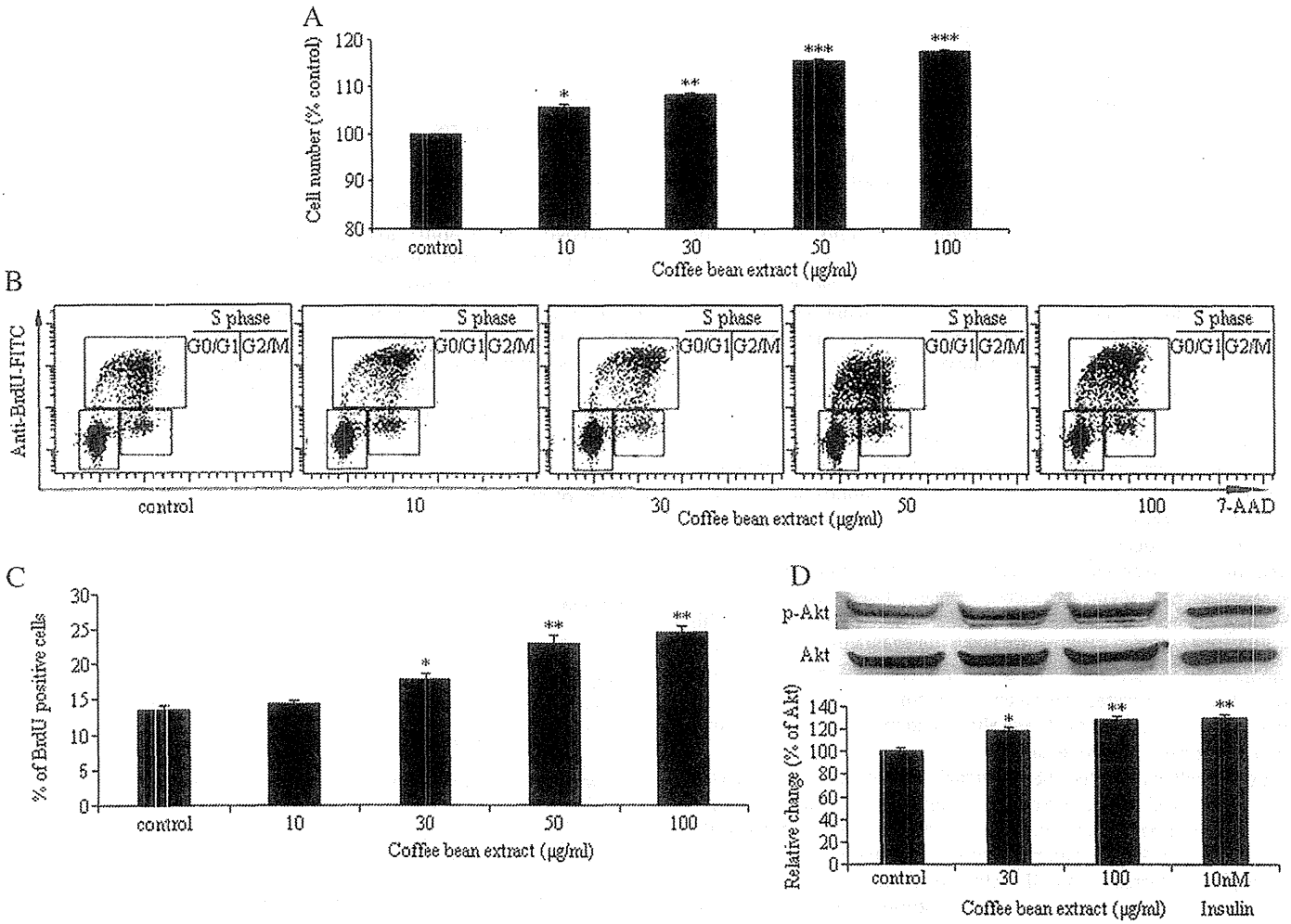

Fig. 4. Effects of coffee on the satellite cells of aged mice in vitro. (A) The satellite cells of aged mice were treated with the indicated concentrations of coffee bean extract for 72 h and the cell proliferation rate was measured. (B) Bromodeoxyuridine (BrdU)/7-AAD incorporation was evaluated by flow cytometry after stimulating the cells with water or indicated concentrations of coffee for 72 h. The regions were set on the G0/G1, S phase and G2/M populations. Representative data are shown. (C) The satellite cells of aged mice were cultured for 72 h with or without the indicated concentrations of coffee, and BrdU-positive percentages were calculated. (D) The satellite cells of aged mice were pretreated with coffee for 72 h, then the western blot analysis detected activated form of Akt (phospho-Akt) and total Akt. The densitometry quantified the band intensities. The graph shows the phospho-Akt band intensities normalized to the Akt band intensities. Representative of 3 independent experiments. Columns are mean ± SE. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.0001$, compared with control.

level of Akt (Fig. 4D). As a positive control, we activated Akt in satellite cells with insulin (Fig. 4D). These results suggested that coffee treatment increased the proliferation rate and augmented DNA synthesis through the Akt signaling pathway in the satellite cells isolated from aged mice in vitro.

4. Discussion

In this study, using aged mice, we showed that coffee treatment increased the skeletal muscle weight, grip strength, regenerating capacity of injured skeletal muscles, and decreased the serum pro-inflammatory mediator levels compared to controls in vivo. In vitro, coffee treatment increased the cell proliferation rate, augmented DNA synthesis, and activated the Akt signaling pathway compared to controls in the satellite cells of aged mice.

Coffee treatment increased the number of proliferating satellite cells isolated from aged mice and augmented their cell cycle compared to controls, which could be the mechanism responsible for the increase in the skeletal muscle weight and grip strength, and accelerated the regeneration of injured skeletal muscles compared to controls. Since these effects possibly antagonized the loss of muscle mass and strength, the results suggested that coffee treatment might improve sarcopenia in aged mice. The coffee-treated group had greater grip strength than the controls. However, comparison between before and after the treatment period within the same groups showed that the grip strength did not change in the coffee-treated groups, whereas the grip strength decreased after the same period in the controls. This result suggested that coffee treatment did not improve but rather attenuated the progression of the decrease in muscle strength. Therefore, the effects of coffee on skeletal muscles might be attenuating the atrophy rather than improving the muscle mass and strength in aged mice. Satellite cells play an essential role in the regeneration of skeletal muscles (Lepper et al., 2011). Although skeletal muscle has the capacity to regenerate itself, this process is not activated in the gradual age-related loss of muscle fibers. The endocrine, autocrine, and paracrine environment in old muscle is less supportive of the activation, proliferation, and differentiation of satellite cells than in young muscle (Welle, 2002). The current results showed that coffee treatment increased the number of proliferating satellite cells, augmented their cell cycle in vivo and in vitro, and accelerated the differentiation and regeneration in vivo. These results suggested that coffee augmented the satellite cell activation. The decreased inflammatory levels by coffee treatment may contribute to the prevention of sarcopenia, as well. The combination of augmented satellite cell activation and decreased inflammatory levels by coffee treatment might antagonize the degenerative environment in old muscles and might prevent the sarcopenia.

Inflammation plays an important role in age-related sarcopenia (Beyer et al., 2012; Jensen, 2008). Therefore, the decreased serum levels of pro-inflammatory mediators after coffee treatment might be one of the mechanisms of the effects of coffee. Results from several experimental studies showed that coffee extracts inhibited inflammation in animal models (J.Y. Kim et al., 2006; Paur et al., 2010). A human clinical trial also demonstrated that coffee intake had beneficial effects on subclinical inflammation (Kempf et al., 2010). Our findings are consistent with these observations. Coffee contains many components and some of them have immunomodulatory effects. For example, a component of coffee, kahweol, inhibited the effect of TNF-α-induced protein and mRNA expression of the adhesion molecules, vascular cell adhesion molecule 1 and intercellular adhesion molecule 1, in human endothelial cells in vitro (H.G. Kim et al., 2006). Kahweol also inhibited the inflammatory response induced by carrageenan in a rat using an acute air pouch inflammation model (J.Y. Kim et al., 2006). These results strongly suggested that some components in coffee have significant anti-inflammatory effects in vitro and in vivo. Moreover, accumulating evidence suggests that coffee is a good source of antioxidant (Svilaas et al., 2004). Because oxidative stress causes inflammation (Butt and Sultan, 2011), the decrease in inflammation by coffee may be partly mediated through the antioxidant mechanism. Furthermore, some studies also reported that caffeine can increase skeletal muscle contractions by increasing calcium ion release (Olorunshola and Achie, 2011). Since this is a study of a complex product, we could not evaluate the effect of each component in the prevention of sarcopenia. However, the results suggested that whole coffee improved sarcopenia in aged mice. Further studies are required to evaluate the effects and mechanisms of the components of coffee on sarcopenia.

In vitro, coffee activated the Akt signaling pathway in satellite cells isolated from aged mice. Since coffee contains a wide variety of components, it is not clear which component(s) activated Akt. However, the effect of coffee on Akt activation is controversial. In several types of cancer cells, coffee decreased the Akt activation level (Choi et al., 2011; Oh et al., 2009). In contrast, in the cells of a Parkinson's disease model, coffee activated Akt and prevented apoptotic cell death (Nakaso et al., 2008). Combined with previous studies, our results suggested that the effect of coffee on Akt activation level might depend on the cell type. Since most satellite cells in aged animals are in a quiescent state, coffee might not activate Akt in vivo. However, satellite cells are activated by increased muscle loading and some of these cells fuse with apparently undamaged myofibers as part of the hypertrophy process (Adams, 2006). Furthermore, a recent study identified apoptotic cells as a new promoter of myoblast fusion (Hochreiter-Hufford et al., 2013). Therefore, some satellite cells might be in an active state in aged animals and coffee treatment might augment their Akt activation in vivo.

Several studies showed that exercise alone did not affect muscle function in aged animal models (Derbre et al., 2012; Leiter et al., 2011, 2012). A previous study indicated that the systemic environment of old animals is a crucial factor for maintaining and improving the function of satellite cells (Brack et al., 2007). In fact, another study suggested that nitric oxide and exercise together promoted muscle function in aged mice (Leiter et al., 2012). Therefore, the effect of exercise on muscle function in aged mice may depend on systemic and/or muscle environments. The decreased systemic inflammatory levels of the aged mice by coffee treatment may be one reason for the discrepancy in the effects on muscle function between exercise and coffee treatment.

Previous studies suggested that caffeine intake increased physical activity and energy metabolism, and decreased body weight (Magkos and Kavouras, 2004). However, in the present study, coffee intake did not change these parameters. The difference in age might partly explain this discrepancy.

A limitation of this study was that we could not clearly rule out whether caffeine, a major biological active component of coffee, or whole coffee itself produced stimulatory effects on proliferation in satellite cells. Several studies have shown that caffeine affected the proliferation rate and activation levels of Akt and inhibited reactive oxygen species in other cell types including epithelial, neuronal, cancer, and vascular smooth muscle cells (Mercer et al., 2012; Miwa et al., 2011, 2013; Nakaso et al., 2008; Sahu et al., 2013; Sarobo et al., 2012). Further study is required to clarify the exact effects and mechanisms of caffeine or other coffee components on the functions of satellite cells isolated from aged mice.

In conclusion, in vivo, coffee treatment increased the muscle weight, grip strength, regenerating capacity of injured muscles, and decreased serum pro-inflammatory mediator levels compared to controls in aged mice. In vitro, coffee increased the cell proliferation rate, augmented the cell cycle, and increased the activation level of the Akt signaling pathway compared to controls in satellite cells isolated from aged mice. These findings suggested that coffee treatment might have a beneficial effect on the prevention of age-related sarcopenia through decreasing the systemic inflammation and activating the Akt signaling pathway in satellite cells.

Conflict of interests

All the authors declare no conflicts of interest to disclose.

References

Adams, G.R., 2006. Satellite cell proliferation and skeletal muscle hypertrophy. Appl. Physiol. Nutr. Metab. 31, 782–790.

Altun, M., Grönholdt-Klein, M., Wang, L., Ulfhake, B., 2012. Senescence. In: Nagata, T. (Ed.), Cellular Degradation Machineries in Age-Related Loss of Muscle Mass (Sarcopenia). Academia.edu, San Francisco, pp. 269–286 (Chapter 13).

Arai, K., Matsuki, N., Ikegaya, Y., Nishiyama, N., 2001. Deterioration of spatial learning performances in lipopolysaccharide-treated mice. Jpn. J. Pharmacol. 87, 195–201.

Asada, M., Ebihara, S., Yamanda, S., Niu, K., Okazaki, T., Sora, I., Arai, H., 2009. Depletion of serotonin and selective inhibition of 2B receptor suppressed tumor angiogenesis by inhibiting endothelial nitric oxide synthase and extracellular signal-regulated kinase 1/2 phosphorylation. Neoplasia 11, 408–417.

Beyer, I., Mets, T., Bautmans, I., 2012. Chronic low-grade inflammation and age-related sarcopenia. Curr. Opin. Clin. Nutr. Metab. Care 15, 12–22.

Brack, A.S., Conboy, M.J., Roy, S., Lee, M., Kuo, C.J., Keller, C., Rando, T.A., 2007. Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis. Science 317, 807–810.

Butt, M.S., Sultan, M.T., 2011. Coffee and its consumption: benefits and risks. Crit. Rev. Food Sci. Nutr. 51, 363–373.

Cai, D., Frantz, J.D., Tawa Jr., N.E., Melendez, P.A., Oh, B.C., Lidov, H.G., Hasselgren, P.O., Frontera, W.R., Lee, J., Glass, D.J., Shoelson, S.E., 2004. IKKbeta/NF-kappaB activation causes severe muscle wasting in mice. Cell 119, 285–298.

Chang, W.C., Chen, C.H., Lee, M.F., Chang, T., Yu, Y.M., 2010. Chlorogenic acid attenuates adhesion molecules upregulation in IL-1beta-treated endothelial cells. Eur. J. Nutr. 49, 267–275.

Choi, M.J., Park, E.J., Oh, J.H., Min, K.J., Yang, E.S., Kim, Y.H., Lee, T.J., Kim, S.H., Choi, Y.H., Park, J.W., Kwon, T.K., 2011. Cafestol, a coffee-specific diterpene, induces apoptosis in renal carcinoma Caki cells through down-regulation of anti-apoptotic proteins and Akt phosphorylation. Chem. Biol. Interact. 190, 102–108.

Clemmons, D.R., 2009. Role of IGF-I in skeletal muscle mass maintenance. Trends Endocrinol. Metab. 20, 349–356.

Cohen, H.J., Pieper, C.F., Harris, T., Rao, K.M., Currie, M.S., 1997. The association of plasma IL-6 levels with functional disability in community-dwelling elderly. J. Gerontol. A Biol. Sci. Med. Sci. 52, M201–M208.

Derbre, F., Gomez-Cabrera, M.C., Nascimento, A.L., Sanchis-Gomar, F., Martinez-Bello, V.E., Tresguerres, J.A., Fuentes, T., Gratas-Delamarche, A., Monsalve, M., Vina, J., 2012. Age associated low mitochondrial biogenesis may be explained by lack of response of PGC-1alpha to exercise training. Age (Dordr.) 34, 669–679.

Ferrucci, L., Harris, T.B., Guralnik, J.M., Tracy, R.P., Corti, M.C., Cohen, H.J., Penninx, B., Pahor, M., Wallace, R., Havlik, R.J., 1999. Serum IL-6 level and the development of disability in older persons. J. Am. Geriatr. Soc. 47, 639–646.

Franco, R., 2008. Coffee and cancer. Med. Clin. (Barc.) 131, 633–635.

Fukada, S., Yamaguchi, M., Kokubo, H., Ogawa, R., Uezumi, A., Yoneda, T., Matev, M.M., Motohashi, N., Ito, T., Zolkiewska, A., Johnson, R.L., Saga, Y., Miyagoe-Suzuki, Y., Tsujikawa, K., Takeda, S., Yamamoto, H., 2012. Hesr1 and Hesr3 are essential to generate undifferentiated quiescent satellite cells and to maintain satellite cell numbers. Development 138, 4609–4619.

Giovannini, S., Marzetti, E., Borst, S.E., Leeuwenburgh, C., 2008. Modulation of GH/IGF-1 axis: potential strategies to counteract sarcopenia in older adults. Mech. Ageing Dev. 129, 593–601.

Greiwe, J.S., Cheng, B., Rubin, D.C., Yarasheski, K.E., Semenkovich, C.F., 2001. Resistance exercise decreases skeletal muscle tumor necrosis factor alpha in frail elderly humans. FASEB J. 15, 475–482.

Guttridge, D.C., Mayo, M.W., Madrid, L.V., Wang, C.Y., Baldwin Jr., A.S., 2000. NF-kappaB-induced loss of MyoD messenger RNA: possible role in muscle decay and cachexia. Science 289, 2363–2366.

Haddad, F., Zaldivar, F., Cooper, D.M., Adams, G.R., 2005. IL-6-induced skeletal muscle atrophy. J. Appl. Physiol. 98, 911–917.
Hawke, T.J., Garry, D.J., 2001. Myogenic satellite cells: physiology to molecular biology. J. Appl. Physiol. 91, 534–551.
Hochreiter-Hufford, A.E., Lee, C.S., Kinchen, J.M., Sokolowski, J.D., Arandjelovic, S., Call, J.A., Klibanov, A.L., Yan, Z., Mandell, J.W., Ravichandran, K.S., 2013. Phosphatidylserine receptor BAI1 and apoptotic cells as new promoters of myoblast fusion. Nature 497, 263–267.
Inoue, I., Yanai, K., Kitamura, D., Taniuchi, I., Kobayashi, T., Niimura, K., Watanabe, T., 1996. Impaired locomotor activity and exploratory behavior in mice lacking histamine H1 receptors. Proc. Natl. Acad. Sci. U. S. A. 93, 13316–13320.
Jensen, G.L., 2008. Inflammation: roles in aging and sarcopenia. JPEN J. Parenter. Enteral Nutr. 32, 656–659.
Kandarian, S.C., Jackman, R.W., 2006. Intracellular signaling during skeletal muscle atrophy. Muscle Nerve 33, 155–165.
Kempf, K., Herder, C., Erlund, I., Kolb, H., Martin, S., Carstensen, M., Koenig, W., Sundvall, J., Bidel, S., Kuha, S., Tuomilehto, J., 2010. Effects of coffee consumption on subclinical inflammation and other risk factors for type 2 diabetes: a clinical trial. Am. J. Clin. Nutr. 91, 950–957.
Kim, J.Y., Kim, D.H., Jeong, H.G., 2006. Inhibitory effect of the coffee diterpene kahweol on carrageenan-induced inflammation in rats. Biofactors 26, 17–28.
Kim, H.G., Kim, J.Y., Hwang, Y.P., Lee, K.J., Lee, K.Y., Kim, D.H., Jeong, H.G., 2006. The coffee diterpene kahweol inhibits tumor necrosis factor-alpha-induced expression of cell adhesion molecules in human endothelial cells. Toxicol. Appl. Pharmacol. 217, 332–341.
Leiter, J.R., Peeler, J., Anderson, J.E., 2011. Exercise-induced muscle growth is muscle-specific and age-dependent. Muscle Nerve 43, 828–838.
Leiter, J.R., Upadhaya, R., Anderson, J.E., 2012. Nitric oxide and voluntary exercise together promote quadriceps hypertrophy and increase vascular density in female 18-mo-old mice. Am. J. Physiol. Cell Physiol. 302, C1306–C1315.
Lepper, C., Partridge, T.A., Fan, C.M., 2011. An absolute requirement for Pax7-positive satellite cells in acute injury-induced skeletal muscle regeneration. Development 138, 3639–3646.
Macaluso, A., De Vito, G., 2004. Muscle strength, power and adaptations to resistance training in older people. Eur. J. Appl. Physiol. 91, 450–472.
Magkos, F., Kavouras, S.A., 2004. Caffeine and ephedrine: physiological, metabolic and performance-enhancing effects. Sports Med. 34, 871–889.
Meng, S.J., Yu, L.J., 2010. Oxidative stress, molecular inflammation and sarcopenia. Int. J. Mol. Sci. 11, 1509–1526.
Mercer, J.R., Gray, K., Figg, N., Kumar, S., Bennett, M.R., 2012. The methyl xanthine caffeine inhibits DNA damage signaling and reactive species and reduces atherosclerosis in ApoE(−/−) mice. Arterioscler. Thromb. Vasc. Biol. 32, 2461–2467.
Miwa, S., Sugimoto, N., Shirai, T., Hayashi, K., Nishida, H., Ohnari, I., Takeuchi, A., Yachie, A., Tsuchiya, H., 2011. Caffeine activates tumor suppressor PTEN in sarcoma cells. Int. J. Oncol. 39, 465–472.
Miwa, S., Yano, S., Torne, Y., Sugimoto, N., Hiroshima, Y., Uehara, F., Mii, S., Kimura, H., Hayashi, K., Efimova, E.V., Fujiwara, T., Tsuchiya, H., Hoffman, R.M., 2013. Dynamic color-coded fluorescence imaging of the cell-cycle phase, mitosis, and apoptosis demonstrates how caffeine modulates cisplatinum efficacy. J. Cell. Biochem. 114, 2454–2460.
Nakaso, K., Ito, S., Nakashima, K., 2008. Caffeine activates the PI3K/Akt pathway and prevents apoptotic cell death in a Parkinson's disease model of SH-SY5Y cells. Neurosci. Lett. 432, 146–150.
Niu, K., Asada, M., Okazaki, T., Yamanda, S., Ebihara, T., Guo, H., Zhang, D., Nagatomi, R., Arai, H., Kohzuki, M., Ebihara, S., 2012. Adiponectin pathway attenuates malignant mesothelioma cell growth. Am. J. Respir. Cell Mol. Biol. 46, 515–523.
Niu, K., Guo, H., Guo, Y., Ebihara, S., Asada, M., Ohrui, T., Furukawa, K., Ichinose, M., Yanai, K., Kudo, Y., Arai, H., Okazaki, T., Nagatomi, R., 2013. Royal jelly prevents the progression of sarcopenia in aged mice in vivo and in vitro. J. Gerontol. A Biol. Sci. Med. Sci. http://dx.doi.org/10.1093/gerona/glt041.
Oh, J.H., Lee, J.T., Yang, E.S., Chang, J.S., Lee, D.S., Kim, S.H., Choi, Y.H., Park, J.W., Kwon, T.K., 2009. The coffee diterpene kahweol induces apoptosis in human leukemia U937 cells through down-regulation of Akt phosphorylation and activation of JNK. Apoptosis 14, 1378–1386.
Okazaki, T., Sakon, S., Sasazuki, T., Sakurai, H., Doi, T., Yagita, H., Okumura, K., Nakano, H., 2003. Phosphorylation of serine 276 is essential for p65 NF-kappaB subunit-dependent cellular responses. Biochem. Biophys. Res. Commun. 300, 807–812.
Okazaki, T., Ebihara, S., Asada, M., Yamanda, S., Saijo, Y., Shiraishi, Y., Ebihara, T., Niu, K., Mei, H., Arai, H., Yambe, T., 2007. Macrophage colony-stimulating factor improves cardiac function after ischemic injury by inducing vascular endothelial growth factor production and survival of cardiomyocytes. Am. J. Pathol. 171, 1093–1103.
Okazaki, T., Ebihara, S., Asada, M., Yamanda, S., Niu, K., Arai, H., 2008. Erythropoietin promotes the growth of tumors lacking its receptor and decreases survival of tumor-bearing mice by enhancing angiogenesis. Neoplasia 10, 932–939.
Okazaki, T., Ni, A., Baluk, P., Ayeni, O.A., Kearley, J., Coyle, A.J., Humbles, A., McDonald, D.M., 2009. Capillary defects and exaggerated inflammatory response in the airways of EphA2-deficient mice. Am. J. Pathol. 174, 2388–2399.
Olorunshola, K.V., Achie, L.N., 2011. Caffeine alters skeletal muscle contraction by opening of calcium ion channels. Curr. Res. J. Biol. Sci. 3, 521–525.
Ong, K.W., Hsu, A., Tan, B.K., 2012. Chlorogenic acid stimulates glucose transport in skeletal muscle via AMPK activation: a contributor to the beneficial effects of coffee on diabetes. PLoS One 7, e32718.
Paur, I., Balstad, T.R., Kolberg, M., Pedersen, M.K., Austenaa, L.M., Jacobs Jr., D.R., Blomhoff, R., 2010. Extract of oregano, coffee, thyme, clove, and walnuts inhibits NF-kappaB in monocytes and in transgenic reporter mice. Cancer Prev. Res. (Phila.) 3, 653–663.
Sahu, S., Kauser, H., Ray, K., Kishore, K., Kumar, S., Panjwani, U., 2013. Caffeine and modafinil promote adult neuronal cell proliferation during 48 h of total sleep deprivation in rat dentate gyrus. Exp. Neurol. 248, 470–481.
Sarobo, C., Lacorte, L.M., Martins, M., Rinaldi, J.C., Moroz, A., Scarano, W.R., Delella, F.K., Felisbino, S.L., 2012. Chronic caffeine intake increases androgenic stimuli, epithelial cell proliferation and hyperplasia in rat ventral prostate. Int. J. Exp. Pathol. 93, 429–437.
Sato, Y., Itagaki, S., Kurokawa, T., Ogura, J., Kobayashi, M., Hirano, T., Sugawara, M., Iseki, K., 2011. In vitro and in vivo antioxidant properties of chlorogenic acid and caffeic acid. Int. J. Pharm. 403, 136–138.
Schaap, L.A., Pluijm, S.M., Deeg, D.J., Visser, M., 2006. Inflammatory markers and loss of muscle mass (sarcopenia) and strength. Am. J. Med. 119 (526), e9–e17.
Shen, T., Park, Y.C., Kim, S.H., Lee, J., Cho, J.Y., 2010. Nuclear factor-kappaB/signal transducers and activators of transcription-1-mediated inflammatory responses in lipopolysaccharide-activated macrophages are a major inhibitory target of kahweol, a coffee diterpene. Biol. Pharm. Bull. 33, 1159–1164.
Sriram, S., Subramanian, S., Sathiakumar, D., Venkatesh, R., Salerno, M.S., McFarlane, C.D., Kambadur, R., Sharma, M., 2011. Modulation of reactive oxygen species in skeletal muscle by myostatin is mediated through NF-κB. Aging Cell 10, 931–948.
Strasser, E.M., Wessner, B., Roth, E., 2007. Cellular regulation of anabolism and catabolism in skeletal muscle during immobilisation, aging and critical illness. Wien. Klin. Wochenschr. 119, 337–348.
Svilaas, A., Sakhi, A.K., Andersen, L.F., Svilaas, T., Strom, E.C., Jacobs Jr., D.R., Ose, L., Blomhoff, R., 2004. Intakes of antioxidants in coffee, wine, and vegetables are correlated with plasma carotenoids in humans. J. Nutr. 134, 562–567.
Tsujinaka, T., Fujita, J., Ebisui, C., Yano, M., Kominami, E., Suzuki, K., Tanaka, K., Katsume, A., Ohsugi, Y., Shiozaki, H., Monden, M., 1996. Interleukin 6 receptor antibody inhibits muscle atrophy and modulates proteolytic systems in interleukin 6 transgenic mice. J. Clin. Invest. 97, 244–249.
Uezumi, A., Fukada, S.I., Yamamoto, N., Takeda, S., Tsuchida, K., 2010. Mesenchymal progenitors distinct from satellite cells contribute to ectopic fat cell formation in skeletal muscle. Nat. Cell Biol. 12, 143–153.
United Nations, 2009. Department of Economic and Social Affairs Population Division: World Population Ageing 2009. United Nations, New York 11.
Visser, M., Pahor, M., Taaffe, D.R., Goodpaster, B.H., Simonsick, E.M., Newman, A.B., Nevitt, M., Harris, T.B., 2002. Relationship of interleukin-6 and tumor necrosis factor-alpha with muscle mass and muscle strength in elderly men and women: the Health ABC Study. J. Gerontol. A Biol. Sci. Med. Sci. 57, M326–M332.
Welle, S., 2002. Cellular and molecular basis of age-related sarcopenia. Can. J. Appl. Physiol. 27, 19–41.
Yamanda, S., Ebihara, S., Asada, M., Okazaki, T., Niu, K., Ebihara, T., Koyanagi, A., Yamaguchi, N., Yagita, H., Arai, H., 2009. Role of ephrinB2 in nonproductive angiogenesis induced by Delta-like 4 blockade. Blood 113, 3631–3639.

and risk of lobar hemorrhage to be identified but is an indication of an association between occurrence of SCLH and different apoA-1 plasma levels. Data regarding apoA-1 as a risk factor for SCLH could be more adequately acquired using a prospective study design. Second, statistical power was poor because of the small sample size.

If these findings are confirmed in further studies with larger samples and different designs, apoA-1 plasma level could become a promising diagnostic and predictive marker of CAA, as well as a potential target for prevention strategies in CAA-related hemorrhage.

Massimiliano Godani, MD
Neurology Unit, Sant'Andrea Hospital, La Spezia, Italy

Roberta Baldi, MD
Elisa Raggio, MD
Epidemiology Unit, Sant'Andrea Hospital, La Spezia, Italy

Elisabetta Traverso, MD
Elisa Giorli, MD
Massimo Del Sette, MD
Neurology Unit, Sant'Andrea Hospital, La Spezia, Italy

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REFERENCES

1. Frank PG, Marcel YL. Apolipoprotein A-1: Structure-function relationships. J Lipid Res 2000;41:853–872.
2. Obici L, Franceschini G, Calabresi L et al. Structure, function and amyloidogenic propensity of apolipoprotein A-1. Amyloid 2006;13:191–205.
3. Lefterov I, Fitz NF, Cronican AA et al. Apolipoprotein A-1 deficiency increases cerebral amyloid angiopathy and cognitive deficits in APP/PS1DeltaE9 mice. J Biol Chem 2010;285:36945–36957.
4. Lewis TL, Cao D, Lu H et al. Overexpression of human apolipoprotein A-1 preserves cognitive function and attenuates neuroinflammation and cerebral amyloid angiopathy in a mouse model of Alzheimer disease. J Biol Chem 2010;285:36958–36968.
5. Handattu SP, Garber DW, Monroe CE et al. Oral apolipoprotein A-1 mimetic peptide improves cognitive function and reduces amyloid burden in a mouse model of Alzheimer's disease. Neurobiol Dis 2009;34:525–534.
6. Charidimou A, Gang Q, Werring D. Sporadic cerebral amyloid angiopathy revisited: Recent insights into pathophysiology and clinical spectrum. J Neurol Neurosurg Psychiatry 2012;83:124–137.
7. Viswanathan A, Greenberg SM. Cerebral amyloid angiopathy in the elderly. Ann Neurol 2011;70:871–880.
8. Yamada M. Predicting cerebral amyloid angiopathy-related intracerebral hemorrhages and other cerebrovascular disorders in Alzheimer's disease. Front Neurol 2012;3:8.

EFFECT OF PRESS NEEDLES ON SWALLOWING REFLEX IN OLDER ADULTS WITH CEREBROVASCULAR DISEASE: A RANDOMIZED DOUBLE-BLIND CONTROLLED TRIAL

To the Editor: The global population is aging.[1] Pneumonia is a leading cause of death in elderly adults, especially those with dysphasia and microaspiration.[2] Because aspiration pneumonia is related to impairment of the swallowing and cough reflex,[3] the development of preventive strategies is needed to improve such protective reflexes to reduce the incidence of pneumonia in older people. In Japan, pneumonia is the third leading cause of death.[4] It was previously reported that acupuzncture at two points on the legs (ST36 and KI3) improved the swallowing reflex after stroke[5] and reduced pharyngeal retention and aspiration.[6]

To investigate the effectiveness of acupuncture with press needles in improving the swallowing reflex in elderly adults with cerebrovascular disease, a three-arm randomized double-blind controlled multicenter trial was conducted by incorporating sham patches and sham points. Individuals aged 65 and older who had had a stroke and had dysphasia were recruited consecutively from two hospitals and two nursing homes in Sendai, Japan. The purpose and design of the study were explained to each individual, and informed consent was obtained. The Tohoku University ethics committee approved the study protocol.

Twenty-nine individuals (10 men, 19 women; mean age ± SD 82.2 ± 7.1) were recruited and assigned randomly to three groups: group 1, press needles (Pyonex; Seirin Corporation, Shizuoka, Japan) on the ST36 and KI3; group 2, sham patches on the acupuncture points; and group 3, press needles on sham points. The needles were 0.2 mm in diameter and 0.6 mm long. The design of the sham patch was identical to that of the press needle except most of the needle had been cut off, so that only the head of the needle remained in the resin. Patches were applied and changed every day for 4 weeks. Latent time of swallowing reflex (LTSR), plasma substance P (SP), Barthel Index, Mini-Mental State Examination, and days of fever were measured at baseline and 4 weeks later.

Table 1 shows the outcomes of this study. The primary outcome was change in LTSR. A statistically significant shortening of LTSR was evident in Group 1 (6.9 ± 2.3 vs 2.5 ± 0.3 seconds, $P = .005$), whereas no statistically significant difference was observed in the other two groups. There was a significant difference between the three groups in change in LTSR ($P = .009$). Change in LTSR in Group 1 was significantly different from that in Group 2 ($P = .008$) but not Group 3 ($P > .99$). There was a significant difference in LTSR at day 28 between Groups 1 and 2 ($P = .001$), but no significant difference between Groups 1 and 3 ($P = .51$). Plasma SP did not change significantly during the study in any group, and no differences in secondary outcomes were observed in any group

Table 1. Primary and Secondary Outcomes

Outcome	Group 1, n = 10	Group 2, n = 10	Group 3, n = 9	Group 1 vs Group 2 *P*-Value[a]	Group 1 vs Group 3 *P*-Value[a]
Primary					
LTSR, seconds, mean (SE)					
Baseline	6.9 (2.3)	4.0 (1.1)	14.9 (6.2)		
Day 28	2.5 (0.3)	8.5 (2.8)	8.4 (3.4)	.008[b]	>.99[b]
P-value	.005[c]	.20[c]	.26[c]		
Plasma substance P, pg/mL, mean (SE)					
Baseline	709.4 (297.9)	809.5 (117.7)	483.4 (129.1)		
Day 28	643.0 (245.2)	801.6 (104.2)	550.4 (121.2)		
P-value	.37[c]	.91[d]	.77[d]		
Secondary outcomes					
Barthel Index, mean (SE)					
Baseline	15.5 (10.2)	17.5 (7.6)	8.3 (7.1)		
Day 28	15.0 (10.4)	18.0 (7.7)	8.9 (7.7)		
P-value	.66[c]	.32[c]	.32[c]		
Mini-Mental State Examination Score, mean (SE)					
Baseline	8.2 (2.8)	5.9 (2.7)	3.7 (2.2)		
Day 28	8.3 (2.9)	6.1 (2.9)	3.7 (2.3)		
P-value	.86[d]	.41[c]	>.99[c]		
Days of fever, mean (SE)					
Baseline	5.0 (3.1)	3.7 (1.5)	1.6 (0.7)		
Day 28	7.0 (3.0)	5.6 (2.4)	1.2 (0.7)		
P-value	.29[c]	.06[c]	.26[c]		
Body mass index, kg/m^2 mean ± SD					
Baseline	19.2 ± 1.4	15.8 ± 3.3	20.2 ± 1.3		
Day 28	16.6 ± 2.6	18.1 ± 3.1	20.3 ± 1.3		
P-value	.17[d]	.10[d]	.67[c]		

SE = standard error.

[a]Intergroup comparisons of change in latent time of swallowing reflex (LTSR).

[b]Kruskal–Wallis test.

[c]Wilcoxon signed rank test.

[d]Paired-*t* test.

between baseline and Day 28. Peripheral blood tests were performed to assess inflammatory and nutritional status. No statistically significant differences were observed in white blood cell count, C-reactive protein, total protein, albumin, or total cholesterol between baseline and Day 28. There were no significant differences in any parameters. Neither press needle nor sham needle treatment caused any side effects.

Attachment of press needles at the two leg acupuncture points improved the swallowing reflex of elderly adults with cerebrovascular disease. Lack of a significant difference between acupuncture points and sham points indicates that the attachment of the fine needle itself has some effect on the swallowing reflex. Even if the press needles are placed at positions that deviate from the acupoints (e.g., sham points in this study), LTSR may improve, which is clinically convenient. This type of acupuncture may become a new adjuvant method for the prevention and treatment of pneumonia in elderly adults.

The limitations of the present study were the small sample size and short follow-up time. In addition, only Group 3 included participants with extremely poor LTSR at baseline, which could have been why there was no difference between Groups 1 and 3 in LTSR at Day 28. Further studies are required to investigate the effect of the press needle on swallowing function.

Akiko Kikuchi, MD, PhD
Department of Traditional Asian Medicine, Graduate School of Medicine, Tohoku University, Miyagi, Japan

Takashi Seki, MD, PhD
Division of Geriatric Behavioral Neurology, Department of Traditional Asian Medicine, Graduate School of Medicine, Tohoku University, Miyagi, Japan

Shin Takayama, MD, PhD
Koh Iwasaki, MD, PhD
Department of Traditional Asian Medicine, Graduate School of Medicine, Tohoku University, Miyagi, Japan

Satoshi Ishizuka, MD, PhD
Sendai Tomizawa Hospital, Miyagi, Japan

Nobuo Yaegashi, MD, PhD
Department of Traditional Asian Medicine, Graduate School of Medicine, Tohoku University, Miyagi, Japan

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