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研究論文● 8

地域在住中高年者・高齢者のエピソード記憶に関する横断的検討

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日本未病システム学会

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研究論文●8

地域在住中高年者・高齢者のエピソード記憶に関する横断的検討

西田裕紀子　　安藤富士子　　下方　浩史*

1. 背景と目的

加齢に伴って「もの忘れが多くなった」「ものが覚えられなくなった」と表現されることがよくある。しかし，記憶システムのすべてが一様に加齢による低下を示すわけではない。高齢になっても比較的維持される記憶と，加齢に影響を受けやすい記憶とがある。

記憶の特性にはいくつかの分類が提唱されており，その1つが意味記憶とエピソード記憶の二分法である[1,2]。意味記憶は一般的な知識に関するもので，例えば，単語の意味や定義などに関する記憶であり，高齢になってもかなり維持される。一方，エピソード記憶は，特定の時に特定の場所で学習された情報に関連する記憶である。例えば，昨日の朝食や，20分前に聞かされた単語リストを想起する場合などで，一般に加齢の影響を受けて低下しやすいと指摘されている。さらに，このエピソード記憶の急激な低下は，アルツハイマー型認知症の初期の記憶障害にも顕著に現れることが明らかになってきており，MCI(mild cognitive impairment)のスクリーニングの手段としての有用性が注目されている[2,3]。病院の「もの忘れ外来」などを受診して，記憶力低下の自覚について不安を訴える高齢者が増加している現状を考えても，どの程度のエピソード記憶の低下が，加齢に伴う通常の変化の範囲にあるのか，あるいは病的な過程の始まりなのかを検討することは急務であり，そのためには，まず，加齢とエピソード記憶との関連を明らかにしておく必要がある。しかしながら本邦では，エピソード記憶を測定する標準化された検査が少なく[4]，十分な数の地域住民を対象とした基礎的資料はほとんど蓄積されていない。

本研究では，地域在住中高年者・高齢者のエピソード記憶に関する横断的な検討を行う。

2. 方法

1．対象

「国立長寿医療センター研究所・老化に関する長期縦断疫学研究(National Institute for Longevity Sciences-Longitudinal Study of Aging(NILS-LSA))」の第4次調査(2004～2006年)に参加した40～86歳の中高年者・高齢者2,345名(平均年齢60.1±12.5歳：男性1,169名，女性1,176名)を対象とした。年代による内訳は，40～49歳572名，50～59歳567名，60～69歳561名，70～79歳533名，80～86歳112名である。平均教育年数は12.3±2.8年で，年代が高くなるほど低く(p trend<0.001)，男性よりも女性の方が低い(p<0.001)。NILS-LSAは，年齢および性で層化無作為抽出された地域住民を対象とした老化と老年病に関する縦断的コホート調査であり，国立長寿医療センター倫理委員会の了承の下に「調査への参加の文書による同意(informed consent)」の得られた者を対象として行われている[5]。

2．エピソード記憶の測定

日本版ウエクスラー記憶検査(WMS-R)[6]の13下位検査のうち，言語性のエピソード記憶を測定する「論理的記憶Ⅰ」「論理的記憶Ⅱ」を施行した。

1）論理的記憶Ⅰ(直後再生)：対象者は2つの短い物

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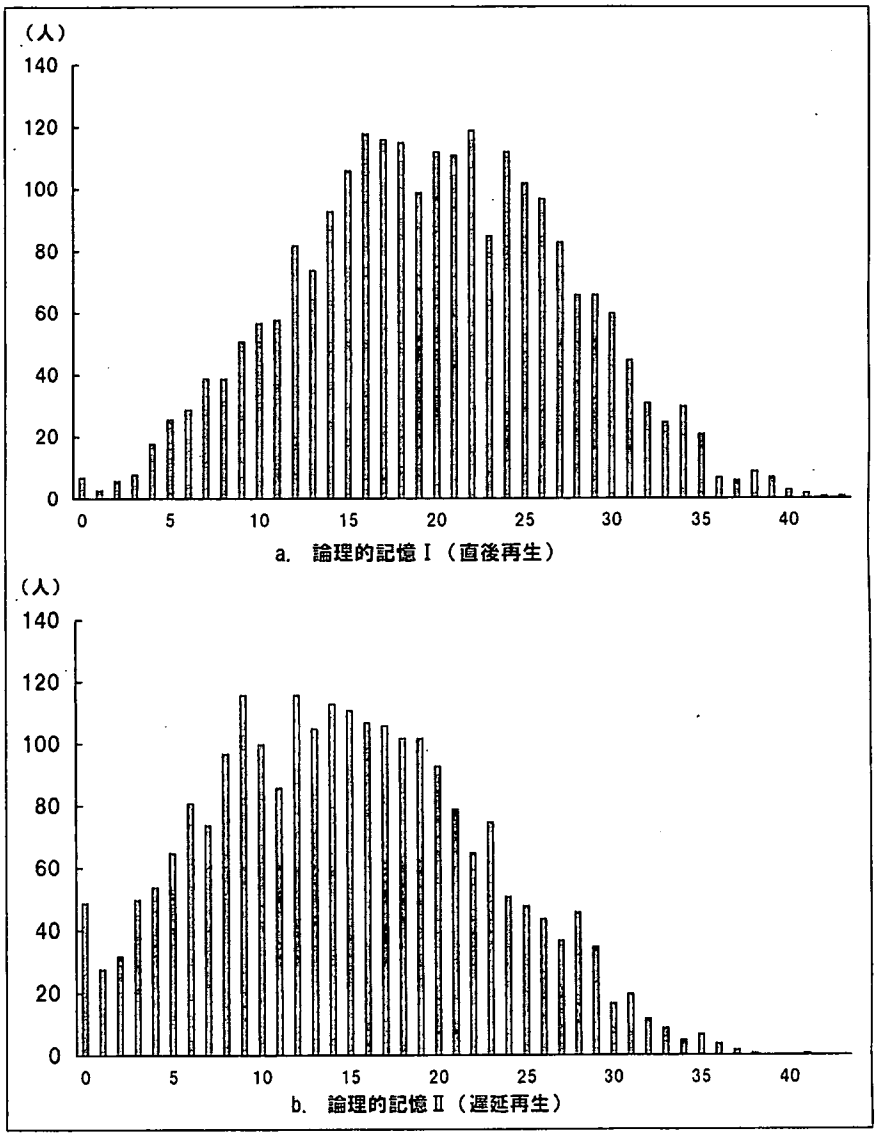

図1　得点分布
注）得点範囲は0～50である。

語を聞く。それぞれの物語を聞いた直後に，覚えていることを再生する。得点範囲は0～50点である。

2）論理的記憶Ⅱ(遅延再生)：「論理的記憶Ⅰ」の終了後，約30分の間隔をあけて，覚えていることを再生する。得点範囲は0～50点である。

3．統計解析

「論理的記憶Ⅰ」「論理的記憶Ⅱ」の各得点を従属変数，年代(40～49/50～59/60～69/70～79/80～)，性(男性/女性)，年代×性の交互作用項を独立変数，教育年数を調整変数とする共分散分析を行った。統計解析にはSAS release 8.2を用いた。

3．結果

1．得点の分布(図1)

対象者の「論理的記憶Ⅰ」「論理的記憶Ⅱ」の得点の分布を図1に示す。平均得点は，「論理的記憶Ⅰ」が19.8±7.6，「論理的記憶Ⅱ」が14.9±7.8であり，分布の左右対称形からの偏りを示す歪度は，「論理的記憶Ⅰ」が0.02，「論理的記憶Ⅱ」は0.24であった。このことは，直後再生と比べて遅延再生では，平均得点が5点程度低下し，得点の分布は負の方向に動くことを示している。

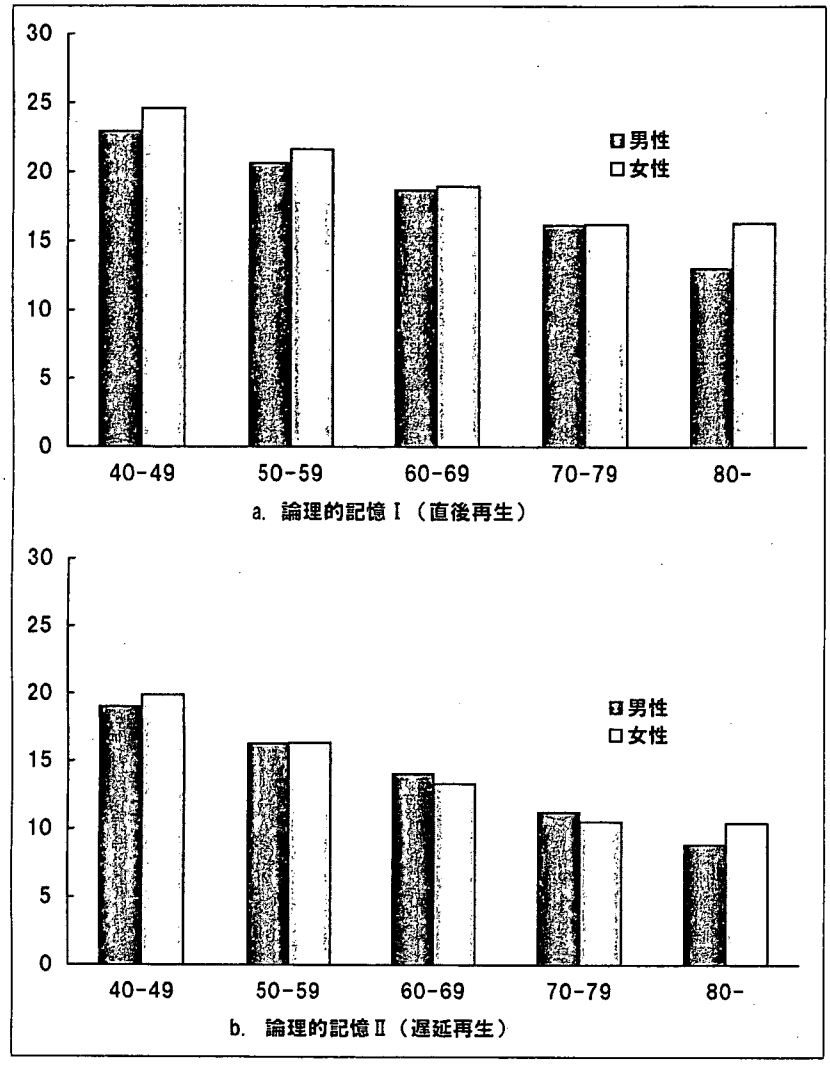

図2　得点分布

注）得点範囲は0～50，教育年数で調整済みの値を示す。

2．年代・性による横断的検討（図2）

共分散分析を行った結果，「論理的記憶Ⅰ」では，年代の主効果が有意であり（$F=94.9$，$p<0.001$），年代が高くなるにつれて得点が低下した。性の主効果も有意で，男性より女性の得点が高かった（$F=10.5$，$p<0.01$）。一方，「論理的記憶Ⅱ」では，年代の主効果が有意であり（$F=114.4$，$p<0.001$），やはり年代が高くなるにつれて得点が低下した。性の主効果は有意ではなかった。

4．考察

地域在住中高年者・高齢者のエピソード記憶は，直後再生，遅延再生ともに，年代が高くなるにつれて低下すること，直後再生では男性よりも女性の得点が高いことが示された。

記憶は，符号化→貯蔵→検索の3つの操作からなる過程である。高齢者の記憶過程では，外界の情報を整理して符号化できないために，貯蔵されたとしても，検索が困難になる場合が多いといわれている[1]。特にエピソード記憶は，一度の個人的な経験に関する記憶であり，意味記憶のように繰り返し学習されるものではないことから，年代が高くなると，体系的，自発的な符号化が難しくなるのではないかと推測される。また，高齢者の記憶の測定には，材料として興味の湧かない情報が提示されたり，新奇の検査場面に緊張したりすることも影響す

る[7]。今回得られた年代差や男女差には，これらが影響していることも考慮する必要がある。

最近では，学習・訓練によってエピソード記憶を維持・向上させることが，アルツハイマー型認知症の発現や進行を防ぐ可能性も指摘されている[8]。今後，さらに経時的な検討を行い，エピソード記憶の個人内変化や，維持・向上に役立つ因子について明らかにする必要がある。

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第48回日本老年医学会学術集会記録
〈市民公開シンポジウム：高齢者の健康と食〉

1. 食生活と長寿

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日本老年医学会雑誌　第44巻　第2号　別刷

第48回日本老年医学会学術集会記録
〈市民公開シンポジウム：高齢者の健康と食〉

1. 食生活と長寿

下方　浩史

要　約　肥満はさまざまな生活習慣病の原因であり，最近はメタボリックシンドロームとしてその病態が注目されている．健康長寿には健全な食生活によって肥満を防止することが重要である．しかしやせているほど健康というわけではなく，人間には理想的な肥満度がある．この理想的な肥満度は年齢によって異なり，高齢者では生命予後を考えた場合，肥満の予防よりもむしろやせの予防の方が重要である．栄養摂取の不足は高齢者では寿命を短くすることが多い．高齢者の栄養のかかえるさまざまな栄養問題，栄養評価に関する考え方を述べるとともに，健康な長寿を目指すための理想的な肥満度，内臓脂肪や体脂肪分布と健康，そして急激な体重変動が健康障害をもたらす等の知見を示し，長寿と食生活，栄養との関連について幅広く紹介する．

Key words：長寿，肥満，食事，老化，栄養

（日老医誌　2007；44：209–211）

はじめに

厚生労働省による平成16年度の簡易生命表では日本人の平均寿命は，女性が85.59歳，男性が78.64歳であった[1]．男女とも5年連続で過去最高を更新したことになる．女性は20年連続の世界一であり，男性は前年の3位から香港を抜いて2位となり，世界最速のペースで長寿化が進んでいる．

日本人の長寿には食生活が重要な要因となっていると考えられる．日本には独特の食習慣がある．先進諸国中で脂肪摂取量が飛び抜けて少なく，米飯を中心として炭水化物の摂取が多い．また魚の摂取が多いことも特徴である．豆腐や納豆，味噌などの大豆製品の摂取が多く，これらは動脈硬化の進行を防ぐには理想に近い食習慣である．またカテキンやビタミンCなどの抗酸化物質が多く含まれる緑茶の摂取は，動脈硬化や癌を防いでいる可能性がある．ここでは長寿や高齢者の健康と栄養との関わりについて述べてみる．

理想的肥満度

自由無制限の食餌を与えたラットより食餌を制限したラットの方が長生きするという結果は基礎老化の研究者の間ではよく知られている[2]．しかし他の動物においても食餌制限が有効かどうかについては議論のあるところで，サルを使ったプロジェクトがアメリカ国立老化研究所で行われつつあるが，サルの寿命は長く最終的な結論がでるのはまだまだ先である[3]．

人間ではやせていればいるほど健康にいいのか．もしそうでないなら，どの程度の体重であるのが医学的には理想なのか．Andresは米国の生命保険会社のデータから，体重（kg）を身長（m）の二乗で割って求めたBody Mass Index（BMI）を身長とは無相関の肥満の指標として用い，各年代で最も死亡率の低いBMIをもとめた[4]．この結果死亡率を縦軸，BMIを横軸にとった時，きれいなU字を描くことに示した．BMIの小さいやせた人では，肺炎や結核などの感染症の発病率が高く，BMIの大きな太った人では糖尿病や心臓病などの発病率が高くなる．男女別に，年齢ごとにこのようなグラフを作成し，死亡率の最も低い肥満度を求めてみると，この理想的な肥満度の値は加齢とともに大きくなっている[5]．男女で大きな差はなく年齢とともにほぼ直線的に理想的なBMIの値が大きくなっていく．例えば，身長170cmの45歳の男性で67kg位の体重であると死亡率，疾患の罹患率が最も低くなる．

日本での検討では，生活習慣病の発生率の最も少ないのは，BMIが22.2であることが示されており[6]，この値は米国の40歳代における最も死亡率の低いBMIの値とほぼ同じ値である．日本人でも，理想的BMIは，米国

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表1　年齢別にみたBMIによる痩せの基準値

年齢	BMI
20～29歳	18
30～39歳	19
40～49歳	20
50～59歳	21
60～69歳	22
70歳以上	23

での場合と同様に，加齢とともに高くなっていると思われるが，残念ながら日本ではこうした加齢による，理想的肥満度の変化についての十分な検討は行われていない．

高齢者の栄養問題

1）老化に伴う生理学的変化

消化吸収という生体機能は原始的機能であり，基本的には予備力が大きい．しかし加齢によって消化吸収に関連する機能は少しずつ低下し，いろいろな疾患や病態を引き起こす．老化により唾液分泌が低下することが多い．唾液が出にくくなれば食物の咀嚼も悪くなる．また食物を飲み込みにくくなり，嚥下障害となる．さらに口腔内の衛生状態も悪くなり，慢性の口内炎や慢性の舌炎，歯槽膿漏の原因となる．口内炎や歯肉炎は入れ歯があわない場合にも起きやすい．口腔内の炎症があれば，不快感や疼痛のため食事が十分とれなくなる．

胃の支持組織の緊張低下により胃液が食道に逆流し，食道にびらんや潰瘍を形成する逆流性食道炎は老人に多い．胃の粘膜が萎縮し胃酸の分泌が悪くなる．鉄やビタミンの吸収が低下し，また胃酸は細菌の増殖を抑える作用があるが，酸が低下すれば消化管への細菌感染の危険が増加する．

消化液の分泌能の低下はとくに油脂類の消化に負担を与える．また歯の脱落や咀しゃく筋の筋力低下による咀しゃく能の低下により，堅い食品を避け，柔らかいものを好むようになる．柔らかい食品には糖質を主体とするものが多く，たんぱく質やカルシウムなどが不足してしまう．消化管の筋組織の筋力低下や支持組織の緊張低下に起因する消化管運動機能の低下によって便秘となりやすい．さらに消化管の栄養素の吸収能，肝臓における処理能力の低下もみられる．このような老化による変化は個人により進行の程度に差はあるとはいえ避けがたいものである．

2）高齢者の食欲不振

高齢者では若年者に比べて食欲が低下することが多い，これにはいくつかの要因がある．高齢者では心肺機能が低下し運動を十分にすることができなくなり，身体活動によるエネルギー消費が少なくなる．運動を行わないため骨格筋が萎縮し体脂肪が増加する．骨格筋は多くのエネルギーを消費するが脂肪組織ではエネルギーはほとんど消費されず，体脂肪率の上昇とともに全身の基礎代謝率は低下する．エネルギー要求量が低くなり，その結果，食欲が低下することが多い．感覚機能，特に食欲に密接に関わる味覚，臭覚，視覚などの機能の低下がいっそう食欲不振を増強させる．高齢者に多い心疾患に対して使われるジギタリス剤などには食欲を減退させる副作用が往々にしてみられる．また亜鉛欠乏は味覚障害を起こし食欲低下の原因となる．

高齢者の栄養状態の評価

1）血液検査による評価

血清アルブミンは高齢者の栄養状態を示す指標として最も有用なものである．健康な高齢者では加齢に伴う血清アルブミンの低下はみられない．血清アルブミンは生命予後の有用な指標でもある[7]．アルブミン値が3.5g/dl以下の状態では骨格筋の消耗が始まっている可能性が強い．高脂血症，特に高コレステロール血症は虚血性心疾患のリスクとなるが，血清コレステロール値が300mg/dlを越えるような場合は家族性の高脂血症であることがほとんどで，治療しない限り老年に達する前に心疾患などで死亡してしまうことが多い．しかし高齢者では低コレステロール血症がむしろ死亡や日常生活の活動能力が低下することにつながることが知られている[8]．

2）体格による評価

高齢者では生命予後を考えた場合，肥満よりもやせの方が重要である．肥満は糖尿病や高血圧の原因のひとつであり，肥満者では心臓病や脳卒中の発生率が高くなる．しかし肥満者の死亡が多いのは主に中年期である．高齢者では中年に比べて肥満は健康を害したりする危険や死亡に結びついたりすることが少ない．表1にBMIでの年齢別にみたやせの基準値を示した[9][10]．年齢が高くなるにつれて基準となるBMIの値が高くなっている．高齢者では椎間の狭小化，椎骨の圧迫骨折による脊椎前弯の増強などにより，身長が年齢とともに低くなっていく．このためBMIは本来あるべき値よりも大きくなっていることにも注意しなければならない．高度の肥満に伴う高血圧症や糖尿病などがないかぎり高齢者に食事制限を勧めるべきではない．高齢者では肥満よりもやせの重要性を認識すべきである．

おわりに

高齢者では一般成人と異なった視点からの栄養管理が必要である．加齢とともに肥満よりもやせのリスクが高くなる．やせた高齢者が寝たきりになると褥創ができやすく，また感染症も治りにくい．低栄養に十分に留意する必要がある．耐糖能は年齢とともに低下する．高齢者で食後血糖やHbA1cが高くなることは高頻度にみられる．40代，50代では糖尿病合併症の進行を抑えるためにも厳格な血糖のコントロールが必要だが，高齢者では過度な制限はむしろ栄養のバランスを崩し，低栄養をきたすこともある．高齢者では血圧も高くなることが多い．しかし食事療法で，無理な減塩を行えば食事が取れなくなってしまい，かえって健康を害することもある．高コレステロールは高齢者ではむしろ生命予後を良くしている．こうした高齢者の特性を考えて，栄養管理を行うことが重要であろう．

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Dietary habit and longevity

Hiroshi Shimokata

Abstract

Obesity is one of the most important causes of life-style related diseases, and recently its pathophysiology is emphasized as metabolic syndrome. Preventing obesity by good dietary habit is a key to achieve healthy longevity. However, a lean body is not always good for health. There is an ideal body size for each person. This ideal body size differs according to age. Especially in the elderly, to prevent weight loss is more important for maintaining health and longevity than to be obese. Malnutrition is a critical factor of diseases and death in the elderly. Problems in nutritional status, and dietary intake, and methods of nutritional assessment in the elderly are discussed. Ideal body size for health and longevity, the relationship of body fat distribution and intra-abdominal fat accumulation health, and the effects of rapid weight change are also discussed to clarify the association of dietary habit and nutrition with longevity.

Key words: *Longevity, Obesity, Diet, Aging, Nutrition*
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ORIGINAL ARTICLE

Is serum albumin a good marker for malnutrition in the physically impaired elderly?

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KEYWORDS
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Summary
Background and Aims: Although serum albumin is well known as a marker of nutritional status, it has remained unclear whether impaired physical function affects serum albumin concentrations in older people. We examined whether hypoalbuminemia can be used as a marker of malnutrition in elderly subjects with various levels of physical impairment.
Methods: A total of 262 elderly subjects without acute illness were enrolled from various geriatric settings. For the nutritional assessment, serum albumin, total cholesterol, anthropometric measurements, and subjective global assessment (SGA) were determined. Physical function was evaluated by rating score of activity of daily living (ADL).
Results: As a whole, participants' serum albumin levels correlated with various nutritional parameters including anthropometric measurements and levels of serum total cholesterol as well as the SGA evaluation. However, after adjusting for age and gender, serum albumin levels in participants with a low ADL function did not correlate with nutritional parameters. Approximately 80% participants with low ADL function who were evaluated as being well nourished according to SGA evaluation had serum albumin levels lower than 35 g/l.
Conclusions: The utility of serum albumin and the traditional cutoff (35 g/l) in older people with low ADL function is questionable even among those without inflammation.

Introduction

Malnutrition is a common finding in the elderly, not only in institutionalized populations but also in community-dwelling

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elderly, with prevalence rates ranging from 12% to 85%.[1,2] Malnutrition is associated with increased hospitalization, increased susceptibility to infection, decreased wound healing, reduced quality-of-life, and increased mortality in the elderly.[3,4]

Multidimensional screening tools such as subjective global assessment (SGA),[5] and anthropometry measurements such as body mass index (BMI), mid-arm circumference (MAC), calf circumference (CC), and skin-fold thickness are generally considered the most easily obtainable, inexpensive, and noninvasive method by which to assess nutritional state. Biochemical measurements such as serum albumin and total cholesterol are also well known as markers for protein energy malnutrition (PEM).[6,7] Among the biochemical parameters, serum albumin levels have long been considered a major measure of malnutrition. On the other hand, some reports have cautioned against using albumin as a measurement of nutritional status in hospitalized patients.[8–10] The criticism is based on the fact that albumin is inversely correlated with markers of inflammatory activity and can behave as an acute-phase reactant, with markedly reduced levels in the setting of acute illness. In addition, it remains unknown whether impaired physical function affects serum albumin concentrations in older people. Thus, we still do not know whether hypoalbuminemia can be used as a marker of malnutrition for elderly people at various levels of activities of daily living (ADL) impairment, especially in the absence of inflammation or acute illness.

In the present study we examined whether hypoalbuminemia defined by a serum albumin level lower than 35 g/l can be used as a marker of malnutrition in elderly subjects without inflammation or acute illness. In addition we also examined whether physical impairment may affect the serum albumin concentration among well-nourished older people.

Subjects and methods

Subjects

We enrolled 262 consecutive elderly subjects (86 males and 176 females, mean age ± SD: 81.8 ± 7.5; range: 65–95 years) from our geriatric outpatient clinic ($n = 69$), a nursing home ($n = 56$), and geriatric hospitals ($n = 72$). Among 262 participants 55 participants were receiving tube feeding and there were no participants receiving parenteral nutrition. The participants from geriatric hospitals were transfers from the acute care setting or from nursing homes for the care of chronic diseases or for the rehabilitation. The nutritional assessments were conducted at the admission. Informed consent for participation, according to procedures approved by the institutional review board of Nagoya University Graduate School of Medicine, was obtained verbally from the patients, or, for those with substantial cognitive impairment, from a surrogate (usually the closest relative or legal guardian) and from caregivers. Subjects diagnosed with infection, inflammation, liver disorders, kidney disorders, cancer at least within 2 months, or serum C-reactive protein ≧1.0 mg/dl were not included among the 262 participants to avoid the influence of inflammation on serum albumin levels.

Anthropometric measurements and biochemical markers

BMI is defined as weight in kg divided by height in meters squared. Triceps skin-fold (TSF) was measured with Harpenden calipers over the triceps muscle at the midway point between the acromion and the olecranon process. MAC and CC were measured on the left arm and calf with a tape measure. Arm muscle circumference (AMC = MAC(cm) $-$ $\pi \times$ TSF(mm)/10) and arm muscle area (AMA) were calculated using the standard formula shown below: AMA $cm^2 = (AMC(cm^2))^2/4\pi$. Three repeat measurements were taken to the nearest 0.5 mm, with the mean taken as the true value. All anthropometric measurements were taken at least twice by two different investigators; the reported values are the means of the repeated measurements. Blood samples were collected after an overnight fast. Serum albumin and total cholesterol levels were determined using automated analyzers.

Nutritional status using SGA was conducted by trained dieticians who were blinded to the levels of serum albumin, total cholesterol, and hemoglobin. SGA consists of a brief nutritional history (weight loss during the last 6 months; dietary change; and a short physical examination of subcutaneous fat, muscle mass, and fluid balance).[5] SGA classifies patients as having PEM or moderate PEM or being well nourished; it focuses on medical issues and was constructed mostly from experience with surgical patients, but the use of SGA in older populations has also been validated.[11]

Each site's nursing staff assessed each patient's functional status which included a rating for seven basic ADL (feeding, bathing, grooming, dressing, using the toilet, walking, and transferring) using summary scores ranging from 0 (total disability) to 20 (no disability).[12] Information obtained from medical records included physician-diagnosed chronic conditions comprising the Charlson comorbidity index,[13] which represents the sum of a weighted index that takes into account the number and seriousness of preexisting comorbid conditions.

Definition of malnutrition

A BMI of less than 20 is widely accepted to indicate that the subject is underweight, particularly in well-developed countries, and 18.5 is recommended as a practical lower limit for most populations.[14] Therefore, a diagnosis of malnutrition was made when BMI was less than 18.5 kg/m^2. Serum albumin and total cholesterol levels were used as the biochemical markers of undernutrition: levels lower than 35 g/l of albumin or 3.88 mmol/l (1.5 g/l) of total cholesterol were taken to indicate malnutrition.[15,16]

Statistical analysis

The ADL score (range 0–20) was categorized into three groups with approximately equal number of participants in each group: high ADL function (ADL score ≧19), mid ADL function (ADL score 2–18), and low ADL function (ADL score <2). Differences between ADL function groups were determined by analysis of variance with a Bonferroni

correction, the χ^2 test, or the Kruskal–Wallis test, as appropriate. Partial rank correlation coefficients adjusted for age and gender were used to measure the relationships between serum albumin levels and anthropometric measurements, biochemical markers, and SGA evaluation. To examine the relationships between ADL scores and serum albumin levels, partial-rank correlation coefficients were used after adjusting for age, gender, and AMC or SGA evaluation. The sensitivity and specificity of 35 g/l of serum albumin as a cutoff point for predicting malnutrition based on the various nutritional markers were also calculated. The significance level was set at 0.05. Data evaluation was carried out using the SPSS software package (SPSS Inc., Chicago, USA).

Results

The age, ADL score, Charlson comorbidity index, anthropometric measurements, serum biochemicals (albumin and total cholesterol), and SGA assessment for total participants and groups categorized by ADL score are shown in Table 1.

The group of low ADL function had the highest comorbidity condition, lowest anthropometric measurements, and lowest levels of serum albumin and total cholesterol compared with the mid or high ADL-function group. Of the low, mid, and high ADL-function groups, 28%, 57.4%, and 87.2% were evaluated as being well nourished according to the SGA classification, respectively.

Among all participants, serum albumin levels were well correlated with various nutritional parameters including anthropometric measurements and the levels of serum total cholesterol as well as SGA classification after adjusting for age and gender (Table 2). Among high and mid ADL-function groups there was also good correlation between serum albumin levels and all nutritional makers tested except for AMA and AMC in the high ADL-function group. However, in the low ADL-function group no correlation was observed between serum albumin level and any nutritional marker tested. Among total participants after adjusting for age, gender and ADL score, serum albumin levels were correlated with BMI ($r = 0.202$, $P = 0.002$), MAC ($r = 0.213$, $P = 0.001$), TSF ($r = 0.265$, $P < 0.0001$), CC ($r = 0.190$, $P = 0.003$), serum total cholesterol ($r = 0.275$, $P < 0.0001$), and SGA classification ($r = 0.288$, $P < 0.0001$) but not with

Table 1 ADL and nutritional characteristics.

	Total, $n = 262$		Low ADL function, ADL score ≦1, $n = 82$		Mid ADL function, ADL score = 2–18, $n = 94$		High ADL function, ADL score ≧19, $n = 86$		P
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Men/women (% of male)	86/176	32.8	29/53	35.4	25/69	26.6	32/54	37.2	0.2666*
Age	81.8	7.5	83.6	8.6	82.5	7.3	79.4	5.7	0.0006
Activities of daily living (ADL, range: 0–20)	10.2	8.7	0.2	0.4	10.3	6.1	19.8	0.4	<0.0001
Charlson index	2.1	1.8	2.6	1.5	2.5	1.9	1.3	1.5	<0.0001
Body mass index (BMI, kg/m^2)	19.7	3.9	17.4	2.8	19.5	3.4	22.2	3.9	<0.0001
Midarm circumference (MAC, cm)	22.2	3.7	20.2	3.3	21.9	3.4	24.6	3.1	<0.0001
Triceps skinfold (TSF, mm)	9.8	5.9	7.2	3.8	8.1	4.1	14.5	6.7	<0.0001
Arm muscle circumference (AMC, cm)	19.1	2.8	17.9	2.7	19.4	2.8	20.0	2.5	<0.0001
Arm muscle area (AMA, cm^2)	29.7	8.6	26.1	7.8	30.6	8.7	32.3	8.1	<0.0001
Calf circumference (CC, cm)	27.0	5.2	22.2	3.3	27.4	3.8	31.7	3.5	<0.0001
Albumin (g/l)	36.0	5.7	31.1	4.0	35.6	4.7	41.0	3.3	<0.0001
Total cholesterol (Tch, mmol/l)	4.8	1.1	4.2	0.9	4.8	1.1	5.3	0.9	<0.0001
Subjective global assessment (n, (% of total))									
Well nourished	152	(58.0)	23	(28.0)	54	(57.4)	75	(87.2)	
Moderatly malnourished	87	(33.2)	42	(51.2)	34	(36.2)	11	(12.8)	<0.0001**
Severely malnourished	23	(8.8)	17	(20.7)	6	(6.4)	0	(0.0)	

Age: high ADL vs. low ADL ($P = 0.0006$) or mid ADL ($P = 0.016$). Charlson index: high ADL vs. low ADL ($P < .0.0001$) or mid ADL ($P < .0.0001$).
BMI, MAC, CC: albumin: high ADL vs. low ADL ($P < 0.0001$) or mid ADL ($P < 0.0001$); mid ADL vs. low ADL ($P < 0.0001$).
TSF: high ADL vs. low ADL ($P < 0.0001$) or mid ADL ($P < 0.0001$).
AMC; high ADL vs. low ADL ($P < 0.0001$), mid ADL vs. low ADL ($P = 0.0012$).
AMA: high ADL vs. low ADL ($P < 0.0001$), mid ADL vs. low ADL ($P = 0.0013$).
Tch: high ADL vs. low ADL ($P < 0.0001$) or mid ADL ($P = 0.011$), mid ADL vs. low ADL ($P < 0.0001$).
SD: Standard deviation.
*χ^2-test.
**Kruskal–Wallis test, others were determined by analysis of variance with a Bonferroni correction.

Table 2 Correlation between serum albumin and neutritional variables.

	Total, $n = 262$		Low ADL function, ADL score ≦1, $n = 82$		Mid ADL function, ADL score = 2–18, $n = 94$		High ADL function, ADL score ≧19, $n = 86$	
	r	P	r	P	r	P	r	P
Body mass index	0.482	<0.0001	0.135	0.2370	0.367	0.0010	0.2391	0.039
Midarm circumference	0.485	<0.0001	0.176	0.1230	0.395	<0.0001	0.2511	0.030
Triceps skinfold	0.501	<0.0001	−0.022	0.8500	0.417	<0.0001	0.3978	<0.0001
Arm muscle circumference	0.297	<0.0001	0.205	0.0710	0.285	0.0090	−0.0335	0.775
Arm muscle area	0.281	<0.0001	0.195	0.0870	0.265	0.0160	−0.0384	0.744
Calf circumference	0.636	<0.0001	0.096	0.4010	0.457	<0.0001	0.2957	0.010
Total cholesterol	0.469	<0.0001	0.194	0.0890	0.394	<0.0001	0.2525	0.029
Subjective global assessment (SGA)	0.499	<0.0001	0.199	0.0810	0.258	0.0190	0.5488	<0.0001

ADL: activities of daily living. Data were adjusted for age and gender.
SGA rating: 0, well nourished; 1, moderatly malnourished; 2, severely malnourished.

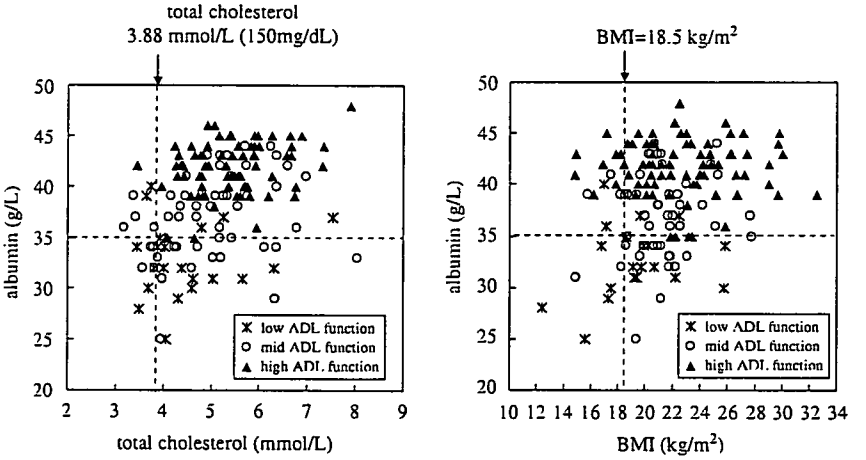

Figure 1 The relationship between levels of serum albumin and total cholesterol or BMI according to the three categories of ADL function among the well-nourished subjects as evaluated by SGA.

AMA ($r = 0.069$, $P = 0.285$) or AMC ($r = 0.086$, $P = 0.183$). Total ADL scores were well correlated with serum albumin concentration after adjusting for gender and age ($r = 0.726$, $P<0.0001$). This correlation persisted after adjusting for SGA classification ($r = 0.650$, $P<0.0001$) or AMC ($r = 0.699$, $P<0.0001$), or both ($r = 0.644$, $P<0.0001$).

Figure 1 shows the relationship between levels of serum albumin and total cholesterol or BMI according to the three categories of ADL function among the subjects evaluated as well nourished by SGA. There were no participants with albumin <35 g/l among the well-nourished high ADL-function group with total cholesterol ≧3.88 mmol/l (150 mg/dl) or BMI ≧18.5 kg/m^2. However, 13 out of 16 participants (81.3%) of the well-nourished low ADL-function group, and 13 out of 44 participants (29.5%) of well-nourished mid ADL-function group had albumin <35 g/l and total cholesterol ≧3.88 mmol/l (150 mg/dl). Furthermore, 12 out of 15 participants (80.0%) of the well-nourished low ADL-function group and 15 out of the 46 participants (32.6%) of the well-nourished mid ADL-function group had albumin <35 g/l and BMI≧18.5 kg/m^2.

In the low ADL-function group, 77.3% of the participants evaluated as being well-nourished according to SGA classification, 78.2% of the participants with serum total cholesterol concentration ≧3.88 mmol/l, and 82.1% of the participants with BMI≧18.5 kg/m^2 had a serum albumin level <35 g/l (Table 3). By contrast, among the high ADL-function group there were no participants with a serum albumin level <35 g/l among those evaluated as being well nourished. Furthermore, only 3.6% of participants with total cholesterol levels ≧3.88 mmol/l and 2.9% of participants with BMI≧18.5 kg/m^2 had serum albumin levels <35 g/l. The sensitivity and specificity of 35 g/l serum albumin as a cutoff point of malnutrition based on the various nutritional markers are presented in Table 3. Among low ADL-function participants with nutritional status based on either SGA evaluation, total cholesterol levels (<3.88 mmol/l), or BMI (<18.5 kg/m^2), the 35 g/l serum albumin cutoff point had

Table 3 Validity of cutoff point of serum albumin (<35 g/l) for malnutritional markers.

Nutritional markers		Serum albumin				P^*	Specificity	Sensitivity
		<35 g/l		≧35 g/l				
		n	%	*n*	%			
Total								
SGA	Well nourished	34	22.8	115	77.2		0.772	
	Moderatly malnourished	57	64.0	32	36.0	<0.0001		
	Severely malnourished	18	78.9	5	21.7			0.783
Tch	≧3.88 mmol/l	73	34.1	141	65.9	<0.0001	0.659	
	< 3.88 mmol/l	35	77.8	10	22.2			0.778
BMI	≧18.5 kg/m^2	45	28.7	112	71.3	<0.0001	0.713	
	< 18.5 kg/m^2	64	62.1	39	37.9			0.621
Low ADL function (*ADL socre:* ≦1)								
SGA	Well nourished	17	77.3	5	22.7	0.421	0.227	
	Moderatly malnourished	34	79.1	9	20.9			
	Severely malnourished	15	88.2	2	11.8			0.882
Tch	≧3.88 mmol/l	43	78.2	12	21.8	0.500	0.218	
	< 3.88 mmol/l	22	84.6	4	15.4			0.880
BMI	≧18.5 kg/m^2	23	82.1	5	17.9	0.787	0.179	
	< 18.5 kg/m^2	43	79.6	11	20.4			0.796
Mid ADL function (*ADL score:* 2–18)								
SGA	Well nourished	17	32.7	35	67.3	0.033	0.673	
	Moderatly malnourished	20	57.1	15	42.9			
	Severely malnourished	3	50.0	3	50.0			0.500
tch	≧3.88 mmol/l	27	36.0	48	64.0	0.003	0.640	
	< 3.88 mmol/l	13	76.5	4	23.5			0.765
BMI	≧18.5 kg/m^2	20	33.9	39	66.1	0.014	0.661	
	< 18.5 kg/m^2	20	60.6	13	39.4			0.606
High ADL function (*ADL socre:* ≧19)								
SGA	Well nourished	0	0.0	75	100.0	<0.0001	1.000	
	Moderatly malnourished	3	27.3	8	72.7			
	Severely malnourished	0		0				
tch	≧3.88 mmol/l	3	3.6	81	96.4	0.947	0.964	
	< 3.88 mmol/l	0	0.0	2	100.0			
BMI	≧18.5 kg/m^2	2	2.9	68	97.1	0.672	0.971	
	< 18.5 kg/m^2	1	6.3	15	93.8			0.063

SGA: subjective global assessment, tch: total cholesterol, BMI: body mass index, ADL: activities of daily living.
*χ^2 test.

high sensitivity (0.882, 0.880, or 0.796, respectively) but low specificity (0.227, 0.218, or 0.179, respectively) as an indicator of malnutrition. Among low ADL-function participants with nutritional status based on SGA evaluation, the 3.88 mmol/l serum total cholesterol as a cutoff point had high specificity (0.727) but low sensitivity (0.500) as an indicator of malnutrition.

Discussion

In the present study we demonstrated that the serum albumin cutoff point of 35 g/l as an indicator malnutrition is not suitable for the elderly with low ADL function. In older people with low ADL function serum albumin levels were not correlated with various nutritional parameters including anthropometric measurements, levels of serum total cholesterol, and SGA evaluation after adjusting for age and gender. Using a serum albumin level <35 g/l as a malnutrition indicator for the ADL-impaired elderly, about 80% of older people without malnutrition would be classified as malnourished (low specificity) while 11–20% of elderly persons with malnutrition would be missed (sensitivity). These results suggest that the use of a serum albumin level <35 g/l as a marker of malnutrition for elderly with low ADL function leads to over-diagnosis of malnutrition. It should be noted that we also observed that the use of a serum total cholesterol level <3.88 mmol/l as a marker of malnutrition would miss the half of the ADL-impaired elderly person with malnutrition.

The observation that serum albumin is a negative acute-phase protein suggests that serum albumin concentration could be a marker of inflammation. In fact, serum levels of albumin decrease in response to acute or chronic inflammation by altering the normal hepatic protein metabolism and inducing capillary leak.[8–10] This concept is responsible for the reports that albumin is not a good marker for the nutritional status of the hospitalized elderly with illness.[17] However, in this study we excluded patients having high C-reactive protein levels or acute illness within the past 2 months. It has been reported that serum albumin levels and SGA, two possible measurements of nutritional status in hospitalized older people, are often discordant.[18] However, this previous interesting report did not address the interaction between serum albumin and the presence of inflammation or ADL status among hospitalized older people.

It has been reported that posture affects serum albumin levels; 1 h in the sitting position after resting in the supine posture during an overnight sleep increases serum albumin by 6.3%.[19] Simply standing upright or sitting increases hydrostatic pressure, and this shift in balance between hydrostatic and oncotic pressures leads to a net movement of fluid from intravascular to interstitial spaces.[20] Most participants with low ADL function in the present study were hospitalized patients, and most of these were bed-ridden elderly. Blood specimens were drawn from low ADL-function participants lying in bed and from high ADL-function ambulatory participants in a sitting position. These postural differences may have affected the serum levels of albumin in both types of participants. However, it has been reported that there is an increase from the lying to the sitting position of about 6.5–7.7% in serum concentrations, not only of proteins but also of lipids including cholesterol.[21,22] Therefore, the posture at the collection of blood samples may not explain our results.

We have demonstrated that ADL function is well correlated with serum albumin levels. One study has demonstrated that severe disability in ADL is strongly associated with anthropometric and biohumoral parameters including serum albumin levels suggesting the presence of malnutrition.[23] However, this is not the case here, since the association between serum albumin and ADL status persists after adjusting for SGA classification, suggesting that this association is not mediated through nutritional status. It is possible that the correlation of serum albumin with ADL function may be mediated by muscle mass, since physical disability is well known to be related with muscle atrophy[24] A cross-sectional study found an association between lower serum albumin concentration and lower muscle mass in the elderly.[25] It is known that several inflammatory cytokines down-regulate serum albumin concentration and increase muscle protein breakdown, which could potentially explain the association of low serum albumin with low muscle mass.[8,26] One study has demonstrated that a low serum albumin concentration in older persons was associated with a greater loss of muscle mass during a 5-year follow-up even after adjusting for the effect of inflammation, although no association was detected between albumin levels and muscle mass at the baseline.[27] In the present study we demonstrated that albumin levels were well correlated with AMC or AMA, markers of muscle mass, among older people without acute illness and inflammation, indicating that inflammation is not involved in the correlation between serum albumin levels and muscle mass, at least in the present study. However, after adjusting for ADL levels there was no correlation between serum albumin and the markers of muscle mass. In addition, the ADL score was well correlated with serum albumin levels after adjusting for muscle mass, suggesting that serum albumin levels might be associated with muscle mass through ADL function rather than with muscle mass directly among older people without acute illness or inflammation. Previous observation has demonstrated that physical exercise increases hepatic synthesis of albumin, resulting in the elevation of plasma albumin content.[28] It is possible that physical activity may be involved in the maintenance of serum albumin concentration through an increase in hepatic synthesis of albumin. Further studies will be required to determine the exact mechanism of the correlation of serum albumin concentration and ADL impairment in well-nourished older people. Since it has been reported that lower serum albumin is independently associated with weaker muscle strength,[29] further research is needed to clarify the exact interactions among serum albumin concentration, ADL status, and not only muscle mass but also muscle strength.

There are limitations in the present study. The distribution of ADL scores of our participants was not the normal distribution. Therefore, no line could be drawn separating the older people with poorer ADL function from those with better ADL function using $<35\,g/l$ of serum albumin as the cutoff point of malnutrition. A limitation included the relative small sample size in each categorized ADL subgroup which may affect the correlation between serum albumin and other nutritional parameters. Another potential limitation of this study was the reliance on self-reported past dietary change and past weight change which are included in SGA in subjects with potential for impaired cognition. We used only anthropometric measurements, AMC and AMC, for assessment of muscle mass; upper arm muscle mass might not reflect the full range of muscle mass.

In the present study we demonstrated that impaired physical function reduced serum albumin concentration even in well-nourished older people. The use of $<35\,g/l$ serum albumin as a marker of malnutrition for the elderly with low ADL function leads to over-diagnosis of malnutrition. Although the exact mechanism of the association between low albumin concentration and disability of ADL function remains unknown, lower muscle mass or decreased physical activity may be involved in this association. Therefore, when nutritional assessment is conducted for older people with impaired ADL function, special attention should be given to the interpretation of results of anthropometric measurements and serum albumin.

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Association of gene polymorphisms with blood pressure and the prevalence of hypertension in community-dwelling Japanese individuals

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Abstract. Hypertension is a complex multifactorial disorder that is thought to result from an interaction between genetic background and environmental factors. Although various loci and genes have been implicated in predisposition to hypertension by genetic linkage analyses and candidate gene association studies, the genes that confer susceptibility to this condition remain to be identified definitively. We examined the relations of nine candidate gene polymorphisms to blood pressure (BP) and the prevalence of hypertension in a population-based study. The 2238 subjects (1110 women, 1128 men) were aged 40 to 79 years and were randomly recruited for a population-based prospective cohort study of aging and age-related diseases in Japan. BP was measured with subjects having rested in a sitting position for at least 15 min. Genotypes for the 160C→T (Arg54Trp) polymorphism of *QPCT*, the C→T (Pro198Leu) polymorphism of *GPX1*, the 137,346T→C polymorphism of *FYN*, the -344C→T polymorphism of *CYP11B2*, and the A→G (Ser49Gly) polymorphism of *ADRB1* were determined with a fluorescence-based allele-specific DNA primer assay system; those for the A→G polymorphism of *CNR2*, the I/D (22,375delAC) polymorphism of *CAV1*, and the -1213T→C polymorphism of *ESR2* by melting curve analysis, and that for the $(GT)_n$ polymorphism of *COL1A2* were determined by DNA fragment analysis. The polymorphism of *FYN* was associated with systolic and diastolic BP in women. In men, polymorphisms of *CNR2*, *QPCT*, *GPX1*, *COL1A2*, *CYP11B2*, and *ESR2* were associated with systolic and diastolic BP, those of *CAV1* and *FYN* with systolic BP, and that of *ADRB1* with diastolic BP. The polymorphisms of *QPCT* and *CYP11B2* were also associated with the prevalence of hypertension in men. These results suggest that polymorphisms of *QPCT* and *CYP11B2* are determinants of BP and the development of hypertension in Japanese men.

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Introduction

Hypertension is a complex multifactorial disorder that is thought to result from an interaction between an individual's genetic background and various environmental factors (1). Given that hypertension is a major risk factor for coronary heart disease, stroke, and chronic renal failure, personalized prevention of hypertension is an important public health goal. One approach to personalized prevention of, and selection of the most appropriate treatment for, hypertension is to identify genes that confer susceptibility to this condition. Although genetic linkage analyses (2-5) and candidate gene association studies (6-9) have implicated various loci and genes in the predisposition to hypertension, the genes that confer genetic susceptibility to this condition remain to be identified definitively. In addition, because of ethnic divergence of gene polymorphisms as well as of environmental factors and lifestyle, it is important to examine polymorphisms related to hypertension in each ethnic group.

We have been attempting to identify genes significantly associated with blood pressure (BP) in Japanese women or men with a population-based approach. In the present study, we selected nine candidate genes that might be expected to contribute to the regulation of BP (Table I) and examined the relations of polymorphisms of these genes to BP, even though there was no apparent biological link among these genes. Our aim was to identify a single polymorphism significantly associated with BP for each gene. Among several polymorphisms previously identified, we selected those that might be expected to affect gene function. We thus examined the relations of these polymorphisms to BP and the prevalence of hypertension in community-dwelling Japanese women and men.

Materials and methods

Study population. The National Institute for Longevity Sciences, the Longitudinal Study of Aging, is a population-

Table I. The nine gene polymorphisms examined in the present study.

Locus	Gene	Symbol	Polymorphism	dbSNP
1p36.11	Cannabinoid receptor 2	*CNR2*	A→G	rs2501431
2p22.2	Glutaminyl-peptide cyclotransferase	*QPCT*	160C→T (Arg54Trp)	rs2255991
3p21.3	Glutathione peroxidase	*GPX1*	C→T (Pro198Leu)	rs1050450
6q21	FYN oncogene related to SRC, FGR, YES	*FYN*	137,346T→C	rs706895
7q22.1	Collagen, type I, α-2	*COL1A2*	$(GT)_n$	ND
7q31.1	Caveolin 1	*CAV1*	I/D (22,375delAC)	rs3840634
8q21-q22	Cytochrome P450, subfamily Y XIB, polypeptide 2	*CYP11B2*	-344C→T	rs1799998
10q24-q26	ß-1-adrenergic receptor	*ADRB1*	A→G (Ser49Gly)	rs1801252
14q23.2	Estrogen receptor 2	*ESR2*	-1213T→C	ND

ND, not detected in dbSNP.

based prospective cohort study of aging and age-related diseases (10). The subjects were unrelated individuals stratified by both age and sex, and were randomly selected from resident registrations in the city of Obu and town of Higashiura in central Japan (11-13). The lifestyle of residents of this area is typical of that of individuals in most regions of Japan. The numbers of men and women recruited were similar and the age at baseline was 40-79 years, with similar numbers of participants in each decade (40, 50, 60 and 70s). The subjects are being followed up every 2 years. All participants were subjected at a special center to a detailed examination, which included not only medical evaluation but also assessment of exercise physiology, body composition, nutrition, and psychology. Individuals with coronary heart disease, valvular heart disease, cardiomyopathies, or renal or endocrinologic diseases that cause secondary hypertension were excluded from the present study. We thus examined the relations of gene polymorphisms to BP or the prevalence of hypertension in 2238 individuals (1110 women, 1128 men). Individuals whose genotypes were not successfully determined were excluded from the analysis. The study protocol complies with the Declaration of Helsinki and was approved by the Committee on Ethics of Human Research of the National Institute for Longevity Sciences. Written informed consent was obtained from each subject.

Measurement of BP. BP was measured with an automatic sphygmomanometer (BP-203RV-II; Colin, Tokyo, Japan) in subjects having rested in a sitting position for at least 15 min. BP in each subject was confirmed with the measurement made by a physician with a mercury manometer according to the guidelines of the American Heart Association (14). Normal BP was defined as both a systolic BP of <140 mmHg and a diastolic BP of <90 mmHg. Hypertension was defined as a systolic BP of ≥140 mmHg or a diastolic BP of ≥90 mmHg (or both), or the use of antihypertensive medication.

Determination of genotype. Genotypes for polymorphisms of *QPCT*, *GPX1*, *FYN*, *CYP11B2*, and *ADRB1* were determined with a fluorescence-based allele-specific DNA primer assay system (Toyobo Gene Analysis, Tsuruga, Japan) (15). Primers and other conditions for genotyping are shown in Table II. The polymorphic region of each gene was amplified by the polymerase chain reaction (PCR) with allele-specific sense primers labeled at the 5' end with either fluorescein isothiocyanate or Texas red and with an antisense primer labeled at the 5' end with biotin. The reaction mixture (25 μl) contained 20 ng of DNA, 5 pmol of each primer, 0.2 mmol/l of each deoxynucleoside triphosphate, 2.5-4 mmol/l $MgCl_2$, and 1 U of rTaq DNA polymerase (Toyobo, Osaka, Japan) in polymerase buffer. The amplification protocol comprised initial denaturation at 95°C for 5 min; 35 cycles of denaturation at 95°C for 30 sec, annealing at 60-70°C for 30 sec, and extension at 72°C for 30 sec; and a final extension at 72°C for 2 min. The amplified DNA was incubated with streptavidin-conjugated magnetic beads in the wells of a 96-well plate at room temperature, and the plate was then placed on a magnetic stand. The supernatants from each well were transferred to the wells of a 96-well plate containing 0.01 mol/l NaOH and were measured for fluorescence with a microplate reader (Fluoroscan Ascent; Dainippon Pharmaceutical, Osaka, Japan) at excitation and emission wavelengths of 485 and 538 nm, respectively, for fluorescein isothiocyanate and of 584 and 612 nm, respectively, for Texas red.

Genotypes for polymorphisms of *CNR2*, *CAV1*, and *ESR2* were determined by melting curve analysis (intercalater-mediated fluorescence resonance energy transfer probe method). The polymorphic region of each gene was amplified by PCR (Table II) in a reaction mixture (25 μl) containing 20 ng of DNA, 5 pmol of each primer, 0.2 mmol/l of each deoxynucleoside triphosphate, 2 mmol/l $MgCl_2$, and 1.25 U of rTaq DNA polymerase in polymerase buffer. The amplification protocol comprised initial denaturation at 95°C for 5 min; 45 cycles of denaturation at 95°C for 30 sec, annealing at 65°C for 30 sec, and extension at 72°C for 30 sec; and a final extension at 72°C for 2 min. A mixture (2 μl) of 10 pmol of probe and SYBR-Green was added to the PCR products, which were then transferred to a PRISM 7700 instrument (Applied Biosystems, Foster City, CA) for measurement of melting temperature. The program for analytic melting comprised incubation at 95°C for 30 sec, 40°C for 1 min, and temperatures increasing to 80°C over 10 min. The fluorescence signals were detected at excitation and emission wavelengths of 485 and 612 nm, respectively.

Table II. Primers, probes, and other PCR conditions for genotyping.

Gene	Polymorphism	Sense primer with FITC	Sense primer with Texas red
QPCT	160C→T (Arg54Trp)	TTTGAATTCATCGGCTCTxCG	TTTGAATTCATCGGCTCTxTG
GPX1	C→T (Pro198Leu)	GCGCCCTAGGCACAGCTxAG	GCGCCCTAGGCACAGCTxGG
FYN	137,346T→C	GGAGTAATTGACAAGGCTCAxCG	AGGAGTAATTGACAAGGCTCAxTG
CYP11B2	-344C→T	TATTAAAAGAATCCAAGGxCC	GTCTATTAAAAGAATCCAAGGxTC
ADRB1	A→G (Ser49Gly)	GAGACAGCGGCTCGGGGxCT	GACAGCGGCTCGGGGxTT

	Antisense primer with biotin	Annealing (°C)	Cycles	Mg^{2+} (mM)	Taq/KOD
QPCT	GGTATCGCTCTATCAGCAATGG	62.5	35	2.5	Taq
GPX1	GTGTGCCCCTACGCAGGTACA	65.0	35	2.5	Taq
FYN	CCTTTCCTCATGCCCCCTAAT	67.5	35	4.0	Taq
CYP11B2	GGACTTTATCTTATCGTGAGATGA	60.0	35	3.0	Taq
ADRB1	GCCGCCCGCCTCGTTG	70.0	35	3.5	Taq

Gene	Polymorphism	Sense primer	Antisense primer
CNR2	A→G	GGGCAGGTAGGAGACTAGTGCTGAGAG	CTCACCCGTGGAAGGGCACTG
CAV1	I/D (22,375delAC)	AAAGGTGATGGATCATTTCCCATTATACAC	TGGGCAATGGTCATCCATGACTG
ESR2	-1213T→C	GAACAGGAGCCAGGGGCACAG	CCTGAAGACAAGTACCTTGCAGCTGAG

	Probe	Annealing (°C)	Cycles	Mg^{2+} (mM)	Taq/KOD
CNR2	CACATGATGCCCAGGGTC	65.0	45	2.0	Taq
CAV1	CAAAATGTGTGTCCATTTCAGG	65.0	45	2.0	Taq
ESR2	AACAGTAAAATTCTGCCTGGG	65.0	45	2.0	Taq

Gene	Polymorphism	Sense primer with FAM	Antisense primer
COL1A2	$(GT)_n$	CAGCACGGTGTCTACCACTGC	ATTACTCCTTAGTATCCACAGTATGTATAC

	Annealing (°C)	Cycles	Mg^{2+} (mM)	Taq/KOD
COL1A2	60.0	35	1.2	KOD

FITC, fluorescein isothiocyanate; FAM, 6-carboxyfluorescein. Oligonucleotide sequences are 5'→3'.

The GT repeats [$(GT)_n$] in the first intron of *COL1A2* were amplified by PCR with a sense primer labeled at the 5' end with 6-carboxyfluorescein and with an antisense primer (Table II). The reaction mixture (25 μl) contained 20 ng of DNA, 5 pmol of each primer, 0.2 mmol/l of each deoxynucleoside triphosphate, 1.2 mmol/l $MgSO_4$, and 0.4 U of KOD plus DNA polymerase (Toyobo) in polymerase buffer. The amplification protocol comprised initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 15 sec, annealing at 60°C for 30 sec, and extension at 68°C for 30 sec; and a final extension at 68°C for 2 min. The size of the $(GT)_n$-containing PCR products was determined with a PRISM 3100 DNA sequencer and with GeneScan and Genotyper software (Applied Biosystems).

Statistical analysis. Age and BP were compared among three groups by one-way analysis of variance and the Tukey-Kramer *post hoc* test, and between two groups by the unpaired Student's t-test. BP values were analyzed in individuals who were not taking antihypertensive drugs. The prevalence of hypertension was compared between two groups (2x2) or among three groups (3x2) by the Chi-square test in all individuals. Allele frequencies were estimated by the gene-counting method, and the Chi-square test was used to identify significant departure from Hardy-Weinberg equilibrium. A P value of <0.05 was considered statistically significant.

Results

Relation of the A→G polymorphism of CNR2 to BP. For men, the distribution of genotypes for the A→G polymorphism of *CNR2* was in Hardy-Weinberg equilibrium and individuals in the combined group of *AG* and *GG* genotypes were younger than those with the *AA* genotype (Table III). Systolic and diastolic BP were significantly higher in men with the *GG* genotype than in those with the *AA* genotype or with the *AG* genotype or in those in the combined group of *AA* and *AG*