表1　対象者の背景因子

No	Age	Sex	CVD	Type of procedure	Coronary risk factors	Weight (kg)	BMI (kg/m²)	$VO_{2@VT}$ (mL/kg/min)	$VO_{2@peak}$ (mL/kg/min)
1	76	F	AP	stent	DL	54.0	23.1	12.1	16.0
2	74	M	MI	stent	HT	63.5	23.6	9.1	18.0
3	56	M	MI	CABG	DM, HT	67.0	23.2	15.6	23.3
4	70	M	MI	stent	HT	56.0	19.2	7.0	19.3
5	72	M	AP	—	HT	69.0	24.2	12.5	17.0
6	66	M	MI	stent	HT, DL	62.0	23.3	18.8	28.2
7	61	M	MI	stent	HT	65.0	23.0	18.8	30.2
8	78	M	MI	stent	IGT	53.0	20.4	11.2	20.8
9	61	F	Paf	ablation	Ob	82.0	32.8	10.8	18.2
10	74	M	—	—	DM, HT, DL, Ob	87.0	33.2	7.9	16.4

Abbreviations. MI：myocardial infarction, AP：angina pectoris, HT：hypertension, DL：dyslipidemia, DM：diabetes mellitus, IGT：impaired glucose tolerance, Ob：Obesity

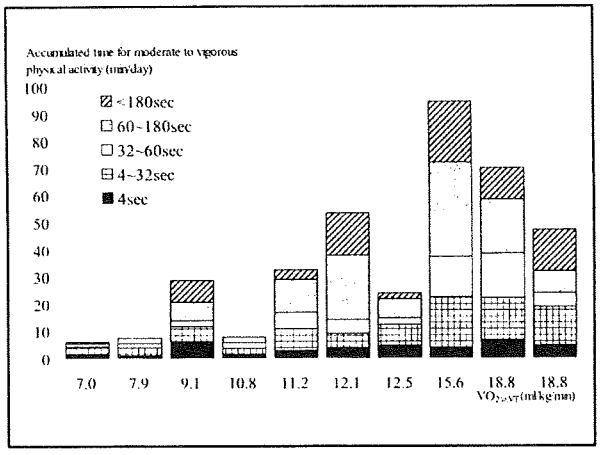

図1　1日あたりの中等度以上の累積身体活動時間

を用いた日常生活での身体活動パターンに関する報告はあるものの，心疾患患者のCPXで測定された運動耐容能と日常生活における中等度以上の身体活動パターンの関連性については不明である．そこで，本研究では4秒ごとの身体活動強度を評価，記録可能な装置を用いて，維持期心臓リハビリテーション教室の参加者における中等度以上の身体活動パターンと運動耐容能の関係について検討した．

方　法

維持期心臓リハビリテーション教室に参加している10名（年齢68±7歳，BMI　24.8±4.6kg/m²）に対して，多メモリー加速度計付歩数計（Lifecorder　EX　4秒版，㈱スズケン，以下，LC_4）を睡眠時と入浴時を除いた終日にわたって，腰部に装着するよう指示した．正しく装着されていたことが確認できた月～金曜日までの連続する5日間のデータを分析した．LC_4は，鉛直方向の加速度の大きさと周期を計測し，4秒ごとの活動強度を独自のアルゴリズムにて0～9の10段階に分類され，その記録が記憶される．先行研究において，LC_4で分類された活動強度と呼気ガス分析によって計測されたトレッドミル歩行時のMETs値との間には高い相関関係が確認されている[9]．本研究では，LC_4が示す10段階の活動強度のうち，「3」以上（≧2.8METs：樋口ら[9]の推定式を用いて算出）を「中等度以上の身体活動」と定義した．同時期に，対象者に対してCPXを実施し，$VO_{2@VT}$と最高酸素摂取量（$VO_{2@peak}$）を求めた．統計学的処理は運動耐容能と中等度以上の累積活動時間との関係をPearsonの相関係数を求めることで検討し，有意水準は5％とした．

結　果

対象者の背景因子を**表1**に示す．CPXによって求めた対象者の$VO_{2@VT}$，および$VO_{2@peak}$はそれぞれ12.3±4.1mL/kg/min（3.5±1.1METs），20.7±4.9mL/kg/min（5.9±1.4METs）であった．LC_4によって評価した中等度以上の累積身体活動時間は37.0±29.2min/dayであり，8秒以上，16秒以上，32秒以上，60秒以上，120秒以上，180秒以上持続して実施された中等度以上の累積身体活動時間は33.6±28.1min/day，29.7±

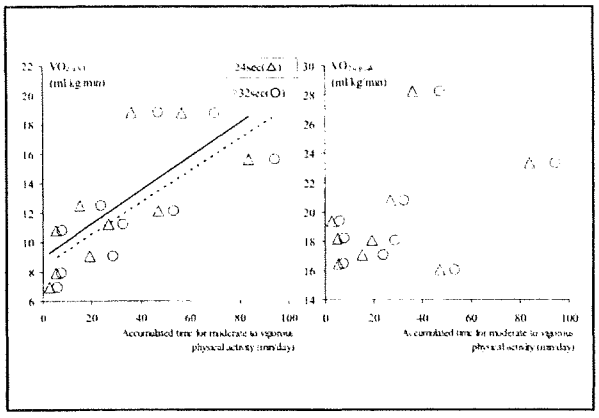

図2　中等度以上の累積身体活動時間と運動耐容能の関係：4秒間以上，または32秒間以上持続した身体活動について

26.2min/day，25.2 ± 23.0min/day，19.6 ± 18.4min/day，11.5 ± 11.6min/day，7.8 ± 8min/day であった．持続時間別の累積時間を対象者別に示したのが**図1**であるが，中等度以上の身体活動は個別にみると短時間で収束している例もあることが明らかになった．中等度以上の累積身体活動時間と $VO_{2@VT}$ との間には**図2**に示すとおり，持続時間の幅に関わらず r = 0.76（≧4秒以上），r = 0.68（≧32秒以上）の有意な正の相関（$p < 0.05$）が認められたが，$VO_{2@peak}$ との間には有意な相関関係は認められなかった．

考　察

本研究では，維持期心臓リハビリテーション教室参加者10名の4秒ごとの身体活動パターンを連続5日間，計10万8千区間を分析した結果，中等度以上の身体活動の頻度や，その水準以上の身体活動を持続して行っている時間には個人差がみられた．さらに，中等度以上の身体活動が持続時間の長短に関わらず累積時間が長い者ほど，CPX によって計測された $VO_{2@VT}$ が高値であることが明らかになった．

国内外で発表されている運動・身体活動ガイドライン[1~4]によれば，運動時間および運動強度については，少なくとも10分間の持続した中等度以上の身体活動を1日合計30分間以上，週5日以上行うことが推奨されており，心臓リハビリテーションプログラムに関するガイドラインにおいても同様の活動指針が出されている[6]．本研究の対象者における LC_4 によって定義された中等度以上の累積活動時間は，5.8~94.4min/day と大きな幅がみられ，さらに5日間の分析期間内に3分以上持続して行われた中等度以上の身体活動が全く無い者も3名存在した．一定強度以上の活動時間と活動持続時間についてアクチグラフを用いて検討された米国女性（年齢39 ± 8歳）を対象とした調査[10]によると，BMI が25未満の者では，過体重者や肥満者にくらべ中等度以上の累積活動時間が長かったことを報告している．米国大学生（年齢20 ± 1歳）を対象にした調査[11]においても，対象学生の53％が1日あたり30分以上の中等度以上の身体活動を行っていたが，10分間以上持続して実施された身体活動に限定した合計活動時間は13.6 ± 12.7min/day と，約96％の学生が条件を満たしていなかったことを報告している．身体機能に問題のない日本人女性（年齢55 ± 11歳）に対して LC_4 を用いて行われた調査[12]においても，日常生活で行われる中等度以上の活動の大半は30秒未満で終了する活動で占められていたと報告されている．比較的低強度の断続的な活動であっても，糖代謝能の維持や体重増加の抑制につながる可能性が示唆されており[13~14]，6年間の積極的な維持期心臓リハビリテーション実施によって冠動脈病変の退縮効果が得られたことを報告した研究[7]においても，介入群には自宅での自転車エルゴメーターでの運動と週2回の集団運動教室参加に加え，運動としての活動量を増やすだけではなく日常的に行う歩行や階段の利用，ガーデニングなどの身体活動を増やすような指導がなされていたことから，個々人の生活に埋め込まれた，短時間で断続的に行われている活動についても注目する意義があると考えられる．

中等度以上の身体活動累積時間と $VO_{2@VT}$ の間には有意な正の相関関係が認められたが，VO_{2peak} との間には得られなかった．我々は日頃 AT 付近の活動強度で生活の大半を過ごしており，日常生活で何気なく行っている家事や普通歩行などの身体活動・運動は3~4METs に相当することから，本研究の対象者の $METs_{@VT}$ が平均3.5METs（範囲：2.0~5.4METs）であり，LC_4 で計測した中等度以上の累積活動時間との相関がみられたことは予想していた結果であった．さらに，本研究の結果より，活動1回あたりの持続時間の長短に関わらず，4秒間以上の累積活動時間が多い者ほど日常活動予備能を規定する $VO_{2@VT}$ が高かったことから，必ずしも10分間以上という持続時間にとらわれず，活動的な生活を過ごすことを推奨するほうが，運動耐容能が低下している者にとって実行可能で有効なアプローチとなりうることを示唆している．近年，身体活動計測装置は収集周期の短

縮，記憶容量の増加などにより，歩数や質問紙による1日単位の身体活動量の評価だけでなく，より細かな時間間隔で身体活動の質・量・パターンを客観的，高精度に計測，評価することが可能になってきているため，身体活動の強度や時間，頻度などの出現パターンと健康，体力に関する諸指標との関連性が今後明らかになってくることが期待される[15]．

本研究は対象者が10名という少人数で，分析対象とした期間も連続した5日間と短期間であったため，今後は対象者を増やして，より長期間の身体活動を分析することや，経時的な身体活動の推移と運動耐容能の関係についても検討が必要と思われる．

結　語

日常生活において中等度以上の身体活動時間を継続的，断続的に関わらず確保することは日常活動予備能を規定する $VO_{2@VT}$ の維持・改善に寄与する可能性が示唆された．

付記

本研究は，東北福祉大学感性福祉研究所における文部科学省の学術フロンティア推進事業（平成16年-平成20年）による私学助成を得て行われた．

文　献

1) 運動所要量・運動指針の策定検討会：健康づくりのための運動基準2006～身体活動・運動・体力～報告書．2006
2) Haskell WL, Lee I-M, Pate RR et al：Physical activity and public health：updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. Circulation 116：1081-1093, 2007
3) U. S. Department of Health and Human Services：2008 Physical Activity Guidelines for Americans, 2008
4) Nelson ME, Rejeski WJ, Blair SN et al：Physical activity and public health in older adults：recommendation from the American College of Sports Medicine and the American Heart Association. Circulation 116：1094-1105, 2007
5) 佐々木保美恵，五安城亜希，澤辺　泰 他：当院における虚血性心疾患患者の運動耐容能の評価．東北理学療法学 18：58-63, 2006
6) Balady GJ, Williams MA, Ades PA et al：Core components of cardiac rehabilitation/secondary prevention programs：2007 update：A scientific statement from the American Heart Association Exercise, cardiac rehabilitation, and prevention committee, the council on clinical cardiology；the councils on cardiovascular nursing, epidemiology and prevention, and nutrition, physical activity, and metabolism；and the American Association of cardiovascular and pulmonary rehabilitation. Circulation 115：2675-2682, 2007
7) Niebauer J, Hambrecht R, Velich T et al：Attenuated progression of coronary artery disease after 6 years of multifactorial risk intervention：role of physical exercise. Circulation 96：2534-2541, 1997
8) Sanada K, Kuchiki T, Miyachi M et al：Effects of age on ventilatory threshold and peak oxygen uptake normalised for regional skeletal muscle mass in Japanese men and women aged 20-80 years. Eur J Appl Physiol 99：475-483, 2007
9) 樋口博之，綾部誠也，進藤宗洋 他：加速度センサーを内蔵した歩数計による若年者と高齢者の日常身体活動量の比較．体力科学 52：111-118, 2003
10) Whitt M, Kumanyika S, Bellamy S：Amount and bouts of physical activity in a sample of African-American women. Med Sci Sports Exerc 35：1887-1893, 2003
11) Dinger MK, Behrens TK.：Accelerometer-determined physical activity of free-living college students. Med Sci Sports Exerc 38：774-779, 2006
12) 綾部誠也，熊原秀晃，青木純一郎 他：4秒毎の加速度計反応を用いた中等度身体活動の継続時間と頻度の評価．肥満研究 13：197-200, 2007
13) Healy GN, Dunstan DW, Salmon J et al：Objectively measured light-intensity physical activity is independently associated with 2-h plasma glucose. Diabetes Care 30：1384-1389, 2007
14) Levine JA, Lanningham-Foster LM, McCrady SK et al：Interindividual variation in posture allocation：possible role in human obesity. Science 307：584-586, 2005
15) Pate RR, O'Neill JR, Lobelo F：The evolving definition of "sedentary". Exerc Sport Sci Rev 36：173-178, 2008

骨髄異形性症候群による高度貧血および心房細動によるうっ血性心不全症例に対するリハビリテーション*

東北大学大学院医学系研究科機能医科学講座
内部障害学分野
金澤　雅之，上月　正博
大崎市民病院内科
南　尚義

症　例

70歳代の男性．数カ月前より自覚していた労作時息切れと下腿浮腫が増悪したため近医循環器内科受診．心機能分類ではNYHA（New York Heart Association）分類のクラスⅢに相当した．高度の貧血と心房細動に起因するうっ血性心不全と診断され入院した．

胸部X線検査では両側に胸水の貯留を認め，心胸郭比は74％であった．心電図検査では心房細動を認めた．血中脳性ナトリウム利尿ペプチドは305 pg/mlと異常高値を示し，心臓超音波検査では軽度ながら僧帽弁と大動脈弁に逆流を認めた．また，左心室壁運動は良好であったが右心系は拡大していた．末梢血のHb値は5.8 g/dlで，骨髄および末梢血検査により骨髄異形性症候群と診断された．

赤血球輸血，強心薬や利尿薬などによる治療が行われて顕性心不全から離脱した．一方，約1カ月間に及ぶ安静臥床により廃用症候群に陥った．

そのため，復職を目指した心臓リハビリテーション（以下，心臓リハ）を行うために当科に紹介され転入院した．

当科入院時の現症と主な検査成績

身長164 cm，体重76 kg，血圧84/56 mmHg．

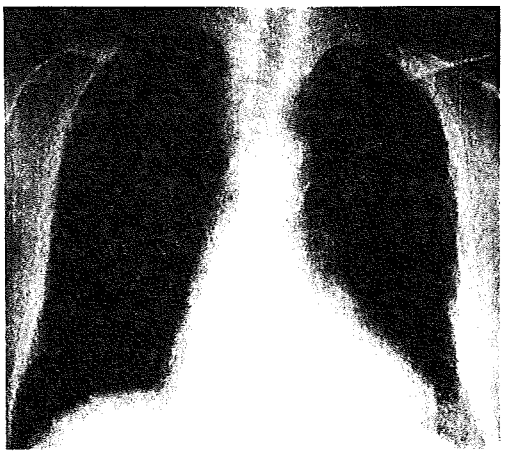

図1　当科入院時の胸部X線像

四肢に麻痺を認めず，筋力は左右の上下肢共に徒手筋力テストで4であった．関節可動域に制限はなく，四肢・体幹に浮腫を認めなかった．Barthel Indexは75点であった．ただしこのスコアはできる日常生活動作の最大値である．

図1に当科入院時の胸部X線像を示す．両側肺野にうっ血所見を認めず，胸水の貯留も認めなかった．心胸郭比は57％であった．

図2に当科入院時の心電図を示す．心拍数90 bpmの心房細動と完全右脚ブロックを認めた．

前医で赤血球輸血を受け，末梢血のHb値は7.6 g/dlに上昇していた．白血球数は3,000/mm^3，血小板数は21.5万/mm^{3}であった．血中脳性ナトリウム利尿ペプチドは251 pg/mlに低下していた．

当科入院中の経過

図3に当科入院中の経過を示す．5月9日に入院し，6月1日に退院した．

入院時にトレッドミル運動負荷試験を行った．3.4 METsまで負荷することが可能であり，その時の血圧は143/51 mmHg，心拍数は169 bpmであった．嫌気性代謝閾値時の運動強度は1.8 METsであり，心拍数は138 bpmであった．試験は下肢疲労で終了した．

運動負荷試験の結果に基づき，運動強度を嫌気性代謝閾値レベルに設定して自転車エルゴメーター運動を開始した．使用した自転車エルゴメー

* 本稿は第45回日本リハビリテーション医学会学術集会パネルディスカッション「リハビリテーション難渋例の実践検討2—呼吸循環器系のハイリスク—」（2008年6月5日，横浜）の講演をまとめたものである．

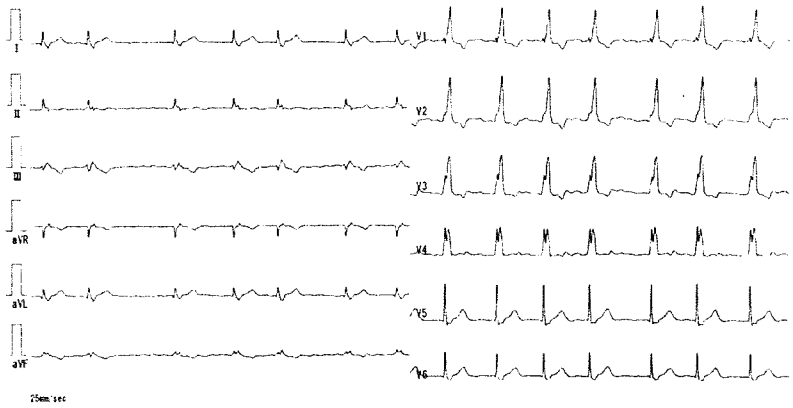

図2　当科入院時の心電図

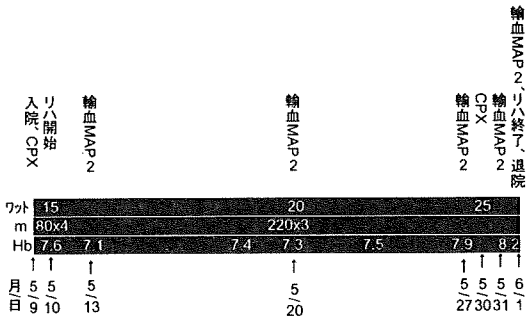

図3　当科入院中の経過

CPX：運動負荷試験，MAP 2：濃厚赤血球2単位，ワット：自転車エルゴメーター運動の強度，m：歩行運動の距離，Hb：末梢血血色素濃度 (g/dl)

ターの最低負荷量である15ワットで運動を開始したが，図4に示すように，約5分間の運動で心拍数が嫌気性代謝閾値レベルを容易に超過するためやむなく運動を休止して心拍数の変化を観察した．約3分間の休息で運動開始前の心拍数にまで低下することが確認されたため，インターバルを3分間とした間欠的運動療法を行うこととした．易疲労性であったため，図3に示すように，開始当初には1日に15ワットで5分間の運動を4クール行うのがやっとであった．5月20日以降には，強度を20ワットに増強し，5クール行うことができるようになった．5月28日以降には強度を25ワットに増強し，5クール行うことが可能となった．

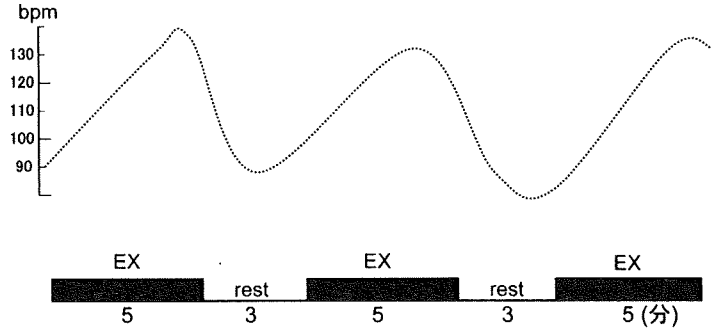

図4　自転車エルゴメーター運動時の運動時間（分）と心拍数 (bpm) との関係

EX：運動，rest：運動休止

歩行運動も連日行った．当初は連続80 mの歩行で心拍数が嫌気性代謝閾値レベルを超えるため，インターバルを挟んで80 mの歩行を1日に4回行った（午前と午後に2回ずつ）．5月20日以降には，距離と回数を増やして行えるようになった．

Hb値を7 g/dl以上に保つために，当科入院中に計5回の濃厚赤血球輸血を行った（図3）．食事は1,900 kcalの食塩7 g制限食とし，内服薬はレニベース®，ダイアート®，アルファロール®，ワーファリン®，胃腸薬を使用した．入院経過中に体重の異常増加や浮腫の出現など，心不全顕性化の徴候や他の有害事象の発生を認めなかった．

当科退院直前に実施したトレッドミル運動負荷試験では，4.0 METsまで負荷することが可能であり，その時の血圧は120/43 mmHg，心拍数は171 bpmであった．嫌気性代謝閾値時の運動強度は2.8 METsであり，心拍数は135 bpmであった．即ち，計24日間にわたる，貧血コントロールを含む心臓リハにより運動耐容能の改善を認めた．

自宅に退院し，事務職に復職した．

リハを行う上で苦労した要因とその対策

骨髄異形性症候群は，骨髄中の造血幹細胞の異常により，無効造血と呼ばれる骨髄での異形性を伴った細胞増殖と，末梢血における血球減少を特徴とする疾患群である．有病率は10万人当たり2.7人で，中高年に多い．単なる難治性の血球減少を来す疾患という性格だけでなく，進行すれば白血病に移行する潜在的悪性性格（前白血病状態）を併せ持つ．治療は，成分輸血（Hb値が6.0～7.0 g/dlで開始．治療目標は自覚症状の消失），免疫抑制療法，サイトカイン療法などである[1]．本症例では，自覚症状がある程度軽減するHb値7 g/dl以上を維持するために，1週間に1回以上の定期的な赤血球輸血が必要であった．

また，本症例は当初，心臓の状態が良くない上に運動療法を行うと，悪い心臓をより一層悪くしてしまうのではないかという強い懐疑心と不安感を持っており，運動療法を行うことに消極的であった．一方で，復職の希望を強く訴えた．そこで，心不全における運動療法の安全性と有効性[2]，復職のために何をなすべきか等に関する情報提供と説得・教育を繰り返し行い，疑いを解き，不安感を和らげるように働きかけた．その結果運動療法を導入し，継続することが可能となった．

本症例の運動療法においては，低強度の運動であっても容易に疲労感が出現し，心拍数が嫌気性代謝閾値レベルを超過した．そのため，心拍数の上昇・下降パターンを考慮して強度・時間・頻度を設定し，下肢を中心とした間欠的運動療法を行った．

間欠的運動とは，あいだに低強度の運動や安静を挟んで短時間の比較的高強度の運動を繰り返すものである．末梢の骨格筋に強い刺激を与える一方で，心血管系に対する危険な高負荷の影響を比較的弱く抑えることが可能なため，近年になり本症例のような高リスク患者に対する有効性と安全性が注目されている．本症例では，高度の貧血を伴う場合のリスク管理の観点から，強度を有酸素運動の範囲内に制限して実施したが，このような低強度の運動でも安全に一定の効果を得ることが可能であったと考える．

おわりに

高度の貧血を合併し，不安感や懐疑心が強いうっ血性心不全症例に対して，貧血のコントロールと共に包括的心臓リハを実施した結果，運動耐容能は改善し，復職することが可能となった．

現時点において，病状が安定しない重症な患者や心筋梗塞以外に起因する心不全患者を対象として，長期間にわたる心臓リハの効果を検討した成績はまだ少ない．今後そのような対象における治療成績が積み重ねられ，心臓リハの適応の拡大が図られることを切に望む．

文　献

1) 近藤敏範，通山　薫：骨髄異形性症候群．日本内科学会雑誌 2006；95：2036-2042
2) 金澤雅之，上月正博：心不全のリハビリテーション．先端医学シリーズ36「リハビリテーション医学の新しい流れ」．先端医療技術研究所，東京，2005；pp 288-293

Green tea consumption is associated with depressive symptoms in the elderly[1–3]

Kaijun Niu, Atsushi Hozawa, Shinichi Kuriyama, Satoru Ebihara, Hui Guo, Naoki Nakaya, Kaori Ohmori-Matsuda, Hideko Takahashi, Yayoi Masamune, Masanori Asada, Satoshi Sasaki, Hiroyuki Arai, Shuichi Awata, Ryoichi Nagatomi, and Ichiro Tsuji

ABSTRACT

Background: Green tea is reported to have various beneficial effects (eg, anti–stress response and antiinflammatory effects) on human health. Although these functions might be associated with the development and progression of depressive symptoms, no studies have investigated the relation between green tea consumption and depressive symptoms in a community-dwelling population.

Objective: The aim of this study was to investigate the relations between green tea consumption and depressive symptoms in elderly Japanese subjects who widely consumed green tea.

Design: We conducted a cross-sectional study in 1058 community-dwelling elderly Japanese individuals aged ≥70 y. Green tea consumption was assessed by using a self-administered questionnaire, and depressive symptoms were evaluated by using the 30-item Geriatric Depression Scale with 2 cutoffs: 11 (mild and severe depressive symptoms) and 14 (severe depressive symptoms). If a participant was consuming antidepressants, he or she was considered to have depressive symptoms.

Results: The prevalence of mild and severe and severe depressive symptoms was 34.1% and 20.2%, respectively. After adjustment for confounding factors, the odds ratios (95% CI) for mild and severe depressive symptoms when higher green tea consumption was compared with green tea consumption of ≤1 cup/d were as follows: 2–3 cups green tea/d (0.96; 95% CI: 0.66, 1.42) and ≥4 cups green tea/d (0.56; 95% CI: 0.39, 0.81) (*P* for trend: 0.001). Similar relations were also observed in the case of severe depressive symptoms.

Conclusion: A more frequent consumption of green tea was associated with a lower prevalence of depressive symptoms in the community-dwelling older population. *Am J Clin Nutr* 2009; 90:1615–22.

INTRODUCTION

Depression in late life is a recognized public health problem. Depression can increase the risk of medical illnesses, worsen the outcome of other medical illnesses, and even increase mortality (1, 2).

Many risk factors are recognized as contributors to the occurrence of depressive symptoms. Stress is particularly well established as a factor that can cause depressive symptoms or contribute to the severity of depression (3). Inflammation also is of key importance for central and peripheral hormonal secretion; it also interacts with neurotransmitters and is related to pathophysiologic processes such as neurodegeneration (4). Epidemiologic studies of patients and community dwellers have shown that inflammatory proteins are associated with depressive symptoms (5).

In Asia, green tea, a widely consumed beverage, has been regarded for centuries to possess significant health-promoting effects (6). Many animal studies have suggested that theanine, one of the major amino acids contained in green tea, has a tranquilizing effect on the brain (7). A laboratory study on acute stress showed that the oral intake of theanine lowered the stress response in human participants (8). Several experimental and animal studies have also shown that green tea is an antiinflammatory agent and that it ameliorates the overproduction of proinflammatory cytokines and mediators (9–11). These effects have been attributed largely to the most prevalent polyphenol contained in green tea, catechin, or flavanol (−) epigallocatechin-3-gallate (12).

Thus, we hypothesized that green tea might have a beneficial effect in the primary and secondary prevention of depressive symptoms or psychological distress due to its antagonistic effects on the stress response and inflammation. However, to the best of our knowledge, only a few studies have reported relations between green tea consumption and mental health (13, 14), and a relation concerning depressive symptoms does not appear to have been investigated. Thus, the relation between green tea

[1] From the Division of Biomedical Engineering for Health and Welfare, Tohoku University Graduate School of Biomedical Engineering, Sendai, Japan (KN, HG, and RN); the Department of Public Health and Forensic Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan (AH, SK, NN, KO-M, HT, and IT); the Department of Geriatrics and Gerontology Division of Brain Sciences, Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan (SE, MA, and HA); the Division of Psychiatry, Kodama Hospital, Sendai, Japan (YM); the Department of Social and Preventive Epidemiology, School of Public Health, The University of Tokyo, Tokyo, Japan (SS); and the Division of Neuropsychiatry and Center for Dementia, Sendai City Hospital, Sendai, Japan (SA).

[2] Supported by a Grant-in-Aid for Scientific Research (no. 13557031) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, by research grants from the Japan Atherosclerosis Prevention Fund, and by a grant for Comprehensive Research on Aging and Health (H18-choju-014) from the Ministry of Health, Labor, and Welfare of Japan.

[3] Address correspondence to K Niu, Division of Biomedical Engineering for Health and Welfare, Tohoku University Graduate School of Biomedical Engineering, 2-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan. E-mail: ggg@mail.tains.tohoku.ac.jp.

Received June 12, 2009. Accepted for publication September 12, 2009.
First published online October 14, 2009; doi: 10.3945/ajcn.2009.28216.

consumption and depressive symptoms in community-dwelling elderly adults, in whom this condition is highly prevalent, remains unclear. In the present study, we investigated the relation between green tea consumption and depressive symptoms in elderly Japanese subjects who consume green tea.

SUBJECTS AND METHODS

Study participants

Our study population comprised subjects aged ≥70 y who resided in the Tsurugaya area of Sendai city, one of the major cities in the Tohoku area of Japan (15, 16). At the time of the study in 2002, there were 2730 individuals aged ≥70 y living in Tsurugaya. All of them were invited to participate in a comprehensive geriatric assessment, which included physical function, cognitive function, and dental status. Of those invited, 1198 participated in the survey and 1178 provided their informed consent for data analysis. The protocol of this study was approved by the Institutional Review Board of the Tohoku University Graduate School of Medicine.

In this study, the depressive symptoms were assessed by using the Geriatric Depression Scale (GDS). Of the 1178 subjects, 1169 completed the GDS (**Figure 1**). Those who did not have any information on diet were excluded (n = 94). Furthermore, subjects who reported cognitive dysfunction (Mini-Mental State Examination score: <18; n = 17) (17) were also excluded. As a result of these exclusions, the final study population comprised 1058 subjects (mean ± SD age: 75.9 ± 4.7 y; men: 42.6%).

Assessment of depressive symptoms

Depressive symptoms were assessed according to the Japanese version (18) of the 30-item GDS. The score ranged from 0 to 30, with greater values indicating increased severity. In this study, 2 cutoffs were used to define different levels of depressive symptoms. The first cutoff was a GDS score ≥11 and/or the use of antidepressants, which indicated relatively mild and severe depressive symptoms. The second cutoff was a GDS score ≥14 and/or the use of antidepressants, indicating relatively severe depressive symptoms.

Assessment of dietary intake

The participants were instructed to fill out a brief self-administered diet-history questionnaire that included 75 food items with specified serving sizes described by natural portions or standard weight and volume measures of the servings commonly

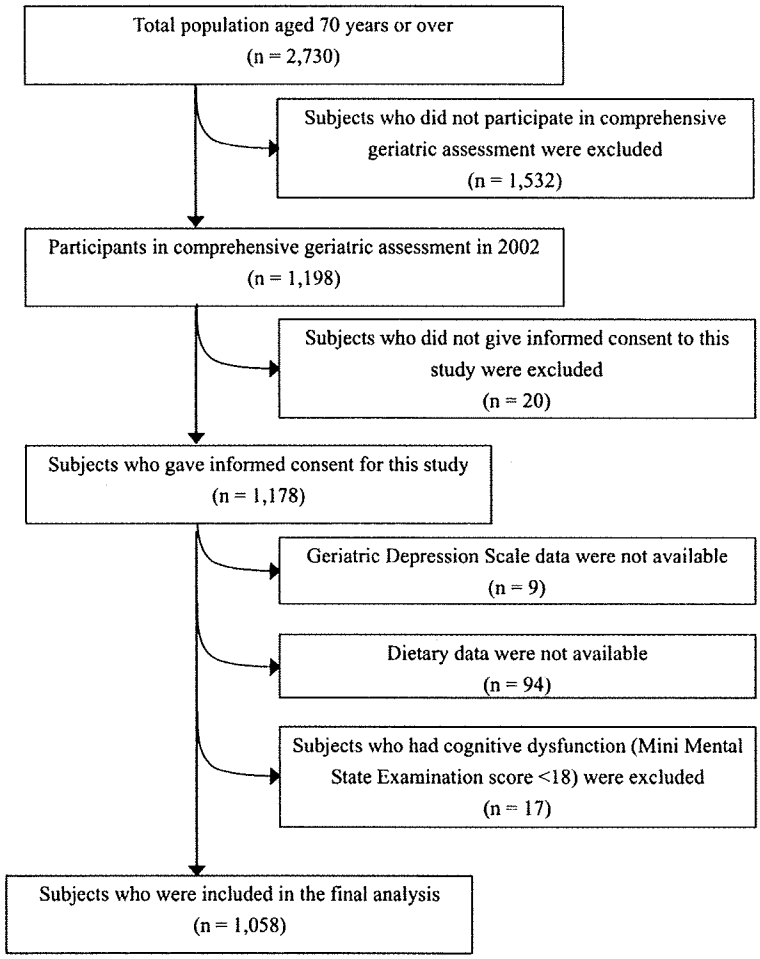

FIGURE 1. Flow chart of the sample selection.

consumed in the study population. The mean daily intake of nutrients was calculated by using an ad hoc computer program developed to analyze the questionnaire. The Japanese food composition tables (19) and others (20) were used as the nutrient database. The reproducibility and validity of the brief self-administered diet-history questionnaire have already been described in detail elsewhere (21).

Participants indicated the mean frequency of consumption of green tea, black or oolong tea, and coffee over the previous 1 mo in terms of the specified serving size by selecting 1 of the 8 frequency categories: almost never, <1 cup/wk, 1 cup/wk, 2–3 cups/wk, 4–6 cups/wk, 1 cup/d, 2–3 cups/d, and ≥4 cups/d. In the study region, the volume of a typical cup of green tea is 100 mL. We summarized these categories in tertile in the following way: green tea (≤1 cup/d, 2–3 cups/d, and ≥4 cups/d), black or oolong tea (almost never, <1 cup/d, and ≥1 cups/d), and coffee (almost never, <1 cup/d, and ≥1 cups/d).

Assessment of other variables

Blood pressure (BP) was measured at home with an HEM747IC device (Omron Life Science Co Ltd, Tokyo, Japan), which uses the cuff oscillometric method to generate a digital display of systolic and diastolic BPs. The mean (±SD) of 15.6 ± 10.4 BP measurements was used as the BP value. Participants who did not measure BP at home on ≥3 d were treated as having missing information on hypertension. Hypertension was defined as a home systolic BP ≥135 mm Hg or a home diastolic BP ≥85 mm Hg or the use of antihypertensive agents (22).

Blood samples were drawn from the antecubital vein, with minimal tourniquet use, while subjects were seated. Specimens were collected in siliconized vacuum glass tubes containing sodium fluoride for blood glucose and no additives for C-reactive protein (CRP) analyses. Blood glucose concentration was measured by using enzymatic methods (Shino-Test, Tokyo, Japan). Diabetes was defined as a casual blood glucose concentration of ≥200 mg/dL or the current use of antidiabetic medication. Highly sensitive CRP concentrations were determined by an immunotechnique that uses a Behring BN II analyzer (Dade Behring, Tokyo, Japan). The BN II assay utilizes a monoclonal antibody coated on polystyrene particles and fixed-time kinetic nephelometric measurements. The detection limit of this assay is 0.02 mg/L. We categorized the study participants on the basis of proposed cutoffs for CRP as low (<1.0 mg/L) or high (at least 1.0 mg/L) (23). The drug information was confirmed by a well-trained pharmacist.

The anthropometric variables (height and body weight) were recorded by using a standard protocol. Body mass index was calculated as weight in kilograms divided by height in meters squared. The sociodemographic variables, which include sex, age, educational level, marital status, cohabitants, perceived social support, and visiting friends, were also assessed. The educational level was assessed by determining the age at completion of schooling and was divided into 2 categories: ≤12 or >12 y (24). Marital status was categorized as follows: married, divorced or widowed, or single. The subjects were also classified as living alone or living with others. Perceived social support (PSS) was evaluated on the basis of the responses (yes or no) to the following 5 questions: "Do you have someone to talk to when you are in trouble?" (PSS1); "Do you have someone to talk to when your physical condition is not good?" (PSS2); "Do you have someone to help you with daily housework?" (PSS3); "Do you have someone to take you to the hospital when you are not feeling well?" (PSS4); and "Do you have someone to take care of you when you are ill and in bed?" (PSS5). These questions were extracted from a previous study on social support and depression among elderly individuals in a rural community (25). A single score was calculated by adding the scores of PSS1–5. The lack of PSS was defined as a PSS score of 0. "Visiting friends" was evaluated on the basis of the responses (yes or no) to the following question: "Do you visit your friends?"

The health-related variables included history of physical illness, pain, cognitive function, instrumental activities of daily living (IADLs), and current medication use. History of physical illness was evaluated on the basis of the responses (yes or no) to questions concerning the history of stroke, ischemic heart disease, cancer, and arthritis. Pain within the previous 4 wk was assessed on the basis of the question, "Have you had any pain recently? If so, how intense was it?" The possible answers were "no pain," "very mild pain," "mild pain," "moderate pain," and "severe pain." Subjects who reported "mild" to "severe" pain were considered to have pain. Cognitive function was assessed with the Mini-Mental State Examination, and scores were classified as belonging to 1 of 3 categories: 18–23, 24–27, and ≥28. The IADL scores were assessed by using the Rouken-Shiki scale (26), and a cutoff of 10/11 was used to determine impairment in IADLs (27).

Information on the smoking ("never," "former," and "current smoking") and drinking ("never," "former," and "current drinking") status of the participants was obtained from a questionnaire survey. Physical activity (PA) was first assessed by a self-reported single question on whether the participant had any PA in the past year. If "yes," further questions were asked about the frequency and duration of walking, brisk walking, and sports. PA was then classified into 3 categories on the basis of the frequency and duration of participation: *1*) "high" (PA ≥3–4 times/wk for ≥30 min each time), *2*) "low" (reporting some PA in the past year, but not enough), and *3*) "none" (no PA). Furthermore, PA was classified into 6 levels on the basis of the above 3 categories and the nature of the physical activity, such as walking, brisk walking, and sports: *1*) level 1 (no walking, brisk walking, or sports), *2*) level 2 (low walking, no brisk walking, no sports), *3*) level 3 (high walking, no brisk walking, no sports), *4*) level 4 (any walking, low brisk walking, no sports), *5*) level 5 (any walking, high brisk walking, no sports), and *6*) level 6 (any walking, any brisk walking, low or high sports). Detailed information has been provided in previous reports (28).

Statistical analysis

The descriptive data have been presented as the mean (with 95% CIs) or as percentages. Depressive symptoms were used as dependent variables, and green tea consumption categories in tertile were used as independent variables. The differences of variables among the green tea consumption categories were examined by analysis of variance for continuous variables or by logistic regression analysis for variables of proportion. For model 1, multiple logistic regression analysis was used to examine relations between green tea consumption and depressive symptoms with adjustment for age; sex; body mass index;

hypertension; diabetes; history of cardiovascular diseases, cancer, or arthritis; high C-reactive protein (≥1.0 mg/L); history of smoking and drinking habits; physical activity (all 6 levels as a categorical variable); cognitive status; impaired IADLs; self-reported body pain; educational level; living alone; and marital status (model 1). For model 2, all of the above variables were used, in addition to serum albumin concentration, total energy intake, intakes per 2000 kcal of energy intake as protein and folate, and consumption frequencies of black or oolong tea (almost never, <1 cup/d, and ≥1 cups/d) and coffee (almost never, <1 cup/d, and ≥1 cups/d). For model 3, all variables in models 1 and 2 in addition to lack of PSS and visiting friends were included. The final multivariate logistic analysis was performed with the forced entry of all factors considered to be potential covariates. Bonferroni-corrected *P* values were used for comparisons between groups differing in green tea consumption. All *P* values for linear trends were calculated by using the categories of green tea consumption (≤1 cup/d: 1; 2–3 cups/d: 2; ≥4 cups/d: 3). The interactions between green tea consumption and all confounders for having depressive symptoms were tested through the addition of the cross-product terms to the regression model. A difference was defined to be significant when $P < 0.05$. All statistical analyses were performed by using the Statistical Analysis System 9.1 edition for Windows (SAS Institute Inc, Cary, NC).

RESULTS

On the basis of the data obtained from 1058 subjects, 34.1% (361/1058) [27.3% (123/451) of men and 39.2% (238/607) of women] were classified as having mild and severe depressive symptoms and 20.2% (214/1058) [14.9% (67/451) of men and 24.2% (147/607) of women] were classified as having severe depressive symptoms.

The participant characteristics according to their green tea consumption status are presented in **Table 1**. The proportion of women, those with a history of cancer, nonsmokers, visiting friends, and widowed (or divorced) status were significantly higher across the green tea consumption tertiles (*P* for trend: <0.0001, 0.04, <0.0001, 0.0001, and 0.02, respectively). The proportion of subjects with a history of cardiovascular disease, who were current smokers or ex-smokers, who were married, and who had impaired IADLs, self-reported body pain, and lack of perceived social support was significantly lower across the categories of green tea consumption (*P* for trend: <0.01, 0.02, <0.0001, <0.01, 0.01, 0.03, and 0.04, respectively). Although the difference was not statistically significant, the proportion of nondrinkers was highest in categories with the lowest green tea consumption. The mean folate consumption ($\mu g \cdot d^{-1} \cdot 2000$ kcal) was significantly higher across categories of green tea consumption (*P* for trend < 0.0001). The mean GDS score was significantly lower across the categories of green tea consumption (*P* for trend < 0.0001). There were no apparent associations between high CRP and green tea consumption. Otherwise, no significant difference was observed between categories of green tea consumption.

The adjusted association between categories of green tea consumption and mild and severe or severe depressive symptoms is shown in **Table 2**. The ORs of the depressive symptoms decreased across categories of green tea consumption. In the final multivariate logistic models, the adjusted ORs for mild and severe depressive symptoms across categories of green tea consumption were 1.00 (reference) for ≤1 cup/d, 0.96 (95% CI: 0.66, 1.42) for 2–3 cups/d, and 0.56 (95% CI: 0.39, 0.81) for ≥4 cups/d (*P* for trend < 0.001). The prevalence of depressive symptoms was 44% lower for participants who consumed ≥4 cups green tea/d tea than for those who consumed ≤1 cup/d (Bonferroni-corrected *P* value <0.01). The ORs of mild and severe depressive symptoms for CRP were 1.00 (reference) for low CRP (<1 mg/L) and 1.08 (95% CI: 0.79, 1.48) for high CRP (≥1.0 mg/L). Similar relations were observed even when we used GDS ≥14 and the use of antidepressants as a definition of depressive symptoms. When we analyzed the relation between the consumption of other beverages and depressive symptoms, a weak or null relation was observed between the consumption of black or oolong tea or coffee and prevalence of depressive symptoms. The multivariate ORs for mild and severe depressive symptoms according to the frequencies of black or oolong tea consumption were 1.00 (reference) for almost never, 0.82 (95% CI: 0.56, 1.20) for <1 cup/d, and 0.71 (95% CI: 0.49, 1.02) for ≥1 cups/d (*P* for trend: 0.06), whereas those for coffee were 1.00 (reference) for almost never, 1.01 (95% CI: 0.73, 1.39) for <1 cup/d, and 0.82 (95% CI: 0.53, 1.27) for ≥1 cups/d (*P* for trend: 0.49). Similar results were also observed when the cutoff ≥14 or the use of antidepressants was used to indicate severe depressive symptoms. Eighteen participants consumed antidepressants in this study. Because individuals who were taking monoamine oxidase inhibitors may have been instructed to avoid the intake of green tea, our findings may have been affected. Therefore, we also analyzed the relations between green tea consumption and depressive symptoms in participants not consuming antidepressants. However, this exclusion did not alter our findings. ORs (95% CI) for mild and severe and for severe depressive symptoms across the green tea consumption tertiles were 1.00, 0.96 (95% CI: 0.67, 1.45), and 0.59 (95% CI: 0.40, 0.87) (*P* for trend < 0.01) and 1.00, 0.97 (95% CI: 0.61, 1.54), and 0.51 (95% CI: 0.32, 0.81) (*P* for trend < 0.01), respectively. We observed a similar relation between green tea consumption and depressive symptoms when men and women were separately analyzed. In model 3, the adjusted ORs (95% CI) for mild and severe and for severe depressive symptoms across the categories of green tea consumption were as follows: for men, the values were 1.00, 0.78 (95% CI: 0.41, 1.48), and 0.45 (95% CI: 0.22, 0.91) (*P* for trend: 0.03) and 1.00, 0.96 (95% CI: 0.44, 2.12), and 0.35 (95% CI: 0.14, 0.87) (*P* for trend: 0.02), respectively; for women, the values were 1.00, 1.09 (95% CI: 0.64, 1.86), and 0.65 (95% CI: 0.40, 1.05) (*P* for trend: 0.04) and 1.00, 0.83 (95% CI: 0.46, 1.49), and 0.50 (95% CI: 0.29, 0.87) (*P* for trend: <0.01), respectively. We did not observe significant interaction between green tea consumption and sex either for mild and severe or for severe depressive symptoms (*P* for interaction: 0.29 for mild and severe and 0.80 for severe). The tests for interaction between the consumption of green tea and other confounders in the final models were also not statistically significant.

DISCUSSION

The present study examined the relation between green tea consumption and depressive symptoms among a community-

TABLE 1
Subject characteristics according to categories of green tea intake[1]

	Categories of green tea intake			
	≤1 cup/d	2–3 cups/d	≥4 cups/d	*P* for trend[2]
n	286	284	488	
Age (y)	75.5 (75.0, 76.1)[3]	76.4 (75.8, 76.9)	75.9 (75.5, 76.3)	0.10
Female sex (%)	48.3	52.8	65.4	<0.0001
BMI (kg/m^2)	23.8 (23.4, 24.2)	23.8 (23.4, 24.2)	24 (23.7, 24.3)	0.80
Serum albumin (g/dL)	4.33 (4.29, 4.36)	4.33 (4.30, 4.36)	4.34 (4.31, 4.36)	0.82
Hypertension (%)	69.6	64.4	70.5	0.61
Diabetes (%)	9.4	8.8	8.8	0.78
History of CVD (%)	19.9	15.9	12.9	<0.01
History of cancer (%)	5.2	4.9	8.8	0.04
History of arthritis (%)	18.5	18.3	17.8	0.80
High CRP (%)[4]	33.9	32.4	31.4	0.46
Smoking status (%)				
Current smoker	16.4	12.7	10.7	0.02
Ex-smoker	39.2	31.0	23.6	<0.0001
Nonsmoker	42.7	55.3	62.9	<0.0001
Drinking status (%)				
Current drinker	41.6	41.2	38.7	0.40
Ex-drinker	14.7	12.0	10.0	0.055
Nondrinker	39.2	44.0	46.3	0.057
PA > level 3 (%)	37.4	41.9	35.3	0.40
Impaired cognitive function (%)				
18 ≤ MMSE < 24	8.4	6.7	7.2	0.58
24 ≤ MMSE < 28	38.5	34.5	34.4	0.29
Impaired IADLs (%)	14.0	15.1	8.4	<0.01
Visiting friends: "yes" (%)	69.6	72.9	81.5	0.0001
Body pain: "yes" (%)	28.0	21.8	20.1	0.01
Lack of perceived social support: total score = 0 (%)	15.7	16.6	10.7	0.03
Educational level ≤12 y (%)	68.2	68.0	71.7	0.26
Living alone: "yes" (%)	22.7	23.9	25.4	0.39
Marital status (%)				
Married	67.1	60.2	59.4	0.04
Widowed or divorced	29.4	34.2	37.5	0.02
Single	3.5	5.6	3.1	0.59
Nutrient intake				
Total energy intake (kcal/d)	1959.9 (1901.3, 2018.5)	2023.9 (1965.2, 2082.7)	1959.6 (1914.8, 2004.4)	0.19
Total protein (g · d^{-1} · 2000 kcal)	82.8 (81.2, 81.2)	81.7 (80.1, 80.1)	83.2 (81.9, 81.9)	0.34
Folate (μg · d^{-1} · 2000 kcal)	336.2 (324.6, 347.8)	372.4 (360.7, 384.1)	404.0 (395.1, 412.9)	<0.0001
GDS scores	9.9 (9.3, 10.5)	9.8 (9.1, 10.4)	8.3 (7.8, 8.8)	<0.0001

[1] CVD, cardiovascular disease; CRP, C-reactive protein; PA, physical activity; MMSE, Mini-Mental State Examination score; IADLs, instrumental activities of daily living; GDS, Geriatric Depression Scale.
[2] Obtained by using ANOVA for continuous variables and logistic regression analysis for variables of proportion.
[3] Mean; 95% CI in parentheses (all such values).
[4] Serum CRP concentrations ≥1.0 mg/L.

dwelling elderly population aged ≥70 y. Our results suggested that high consumption of green tea was significantly related to a lower prevalence of depressive symptoms.

In this large community-based population study, we adjusted for a considerable number of confounding factors. First, we considered that older age, chronic disease, inflammatory status, body mass index, cognitive impairment, disability, lifestyle factors, and psychological problems were potential confounders. However, adjustments for these confounding factors did not change the significant inverse relation between green tea consumption and depressive symptoms. That is, the inverse relation between the frequency of green tea consumption and depressive symptoms was independent of these factors. Second, the effect of the consumption of folate (29) and other beverages such as black or oolong tea or coffee on depressive symptoms was adjusted. Moreover, depressive symptoms can affect hunger and thirst and thus affect nutritional intake (30, 31). Accordingly, we made adjustments for total energy intake, protein consumption, and serum albumin concentration. However, the adjustment for the consumption of these factors also did not change the significant inverse relation between green tea consumption and depressive symptoms. Third, green tea consumption is a unique form of social activity among the Japanese and this, in itself, may influence the depression status. However, the adjustment for perceived social support and visiting friends did not change the significant inverse relation between green tea consumption and depressive symptoms. The association between green tea consumption and the 2 grades (mild and severe and severe) of

TABLE 2
Adjusted relations between consumption of green tea and mild and severe or severe depressive symptoms[1]

	Categories of green tea consumption			
	≤1 cup/d	2–3 cups/d	≥4 cups/d	*P* for trend[2]
n	286	284	488	
No. of mild and severe depressive symptoms, defined as GDS ≥11 or use of antidepressants	114	111	136	—
Model 1[3]	1.00	0.95 (0.66, 1.36)[4]	0.56 (0.40, 0.78)[5]	<0.001
Model 2[6]	1.00	0.96 (0.66, 1.40)	0.54 (0.37, 0.78)[5]	<0.001
Model 3[7]	1.00	0.96 (0.66, 1.42)	0.56 (0.39, 0.81)[5]	0.001
No. of severe depressive symptoms, defined as GDS ≥14 or use of antidepressants	75	67	72	—
Model 1[3]	1.00	0.91 (0.60, 1.37)	0.48 (0.33, 0.71)[5]	<0.001
Model 2[6]	1.00	0.92 (0.59, 1.42)	0.46 (0.30, 0.72)[5]	<0.001
Model 3[7]	1.00	0.92 (0.59, 1.44)	0.48 (0.31, 0.75)[5]	<0.001

[1] GDS, Geriatric Depression Scale.
[2] Obtained by using multiple logistic regression analysis.
[3] Adjusted for age; sex; BMI; hypertension; diabetes; history of cardiovascular diseases, cancer, or arthritis; high C-reactive protein (≥1.0 mg/L); history of smoking and drinking habits; physical activity (all 6 levels as a categorical variable); cognitive status; impaired instrumental activities of daily living; self-reported body pain; educational level; living alone; and marital status.
[4] Adjusted odds ratio; 95% CI in parentheses (all such values).
[5] Significantly different from green tea consumption of ≤1 cup/d, $P < 0.01$ (Bonferroni-corrected).
[6] Additionally adjusted for serum albumin concentration, total energy intake, intakes per 2000 kcal of energy intake as protein and folate, black or oolong tea consumption, and coffee consumption.
[7] Additionally adjusted for lack of perceived social support and visiting friends.

depressive symptoms was tested in this study. Similar relations were observed consistently in the case of both cutoffs. We also conducted a stratified analysis for sex, and similar relations were also observed when men and women were analyzed separately.

In this study, our primary hypothesis was that green tea may have a potentially beneficial effect on the prevention of depressive symptoms due to its anti–stress response and antiinflammatory effects. However, the antiinflammatory mechanisms were less likely to explain our findings. We did not observe any relations between green tea consumption and CRP. CRP also was not associated with depressive symptoms in this elderly population. Thus, CRP did not explain the inverse relation between green tea consumption and depressive symptoms.

We considered that the other mechanism (ie, the anti–stress response effect) of green tea might explain our findings. Theanine might be a candidate for explaining the observed inverse association between green tea consumption and depressive symptoms. Theanine is one of the major amino acid components in green tea and can pass through the blood-brain barrier (32). Dopamine and serotonin dysfunction is a credible etiological candidate for depressive symptoms (33), and animal neurochemistry studies have suggested that theanine increases the brain serotonin and dopamine concentrations (7). Moreover, theanine is also contained in other kinds of tea, such as black or oolong tea (34). In fact, in the current study, a weak, although not statistically significant, relation was also observed between the consumption of black or oolong tea and the prevalence of depressive symptoms (*P* for trend: 0.06). Thus, these data prove a useful hypothesis that higher consumption of green tea is related to a lower prevalence of depressive symptoms, possibly because it leads to a decrease in the stress response. A further study is required to clarify whether green tea or theanine have a beneficial effect on the prevention and treatment of depressive symptoms.

Our recent findings are also consistent with the present findings. Hozawa et al (13) investigated the relation between the frequency of green tea consumption and psychological distress. The study analyzed 42,093 Japanese individuals aged ≥40 y from the general population residing in the rural area of Japan. The study also showed an inverse relation between the frequency of green tea consumption and psychological distress as assessed by K6 (35). The OR and 95% CI of having psychological distress in subjects who consumed ≥5 cups green tea/d was 0.80 (95% CI: 0.70, 0.91) as compared with the subjects who consumed <1 cup green tea/d after adjustment for the possible confounding factors. The inverse association between green tea consumption and mental ill health was consistently observed whether the population was older (the present study) or middle aged (13), whether urban (the present study) or rural (13), whether being assessed by GDS (the present study) or by K6 (13). We considered that these 2 sets of findings corroborate our conclusion that green tea consumption is associated with mental well-being.

This study has several limitations. First, because the assessments were performed in a public facility, the participants were more active and healthy than those who did not undergo the assessment. Therefore, our results might not represent an elderly general population. Second, the GDS is designed for measuring the intensity of depressive symptoms and not for making a clinical diagnosis of depressive episodes. Therefore, a larger population study that uses a standardized comprehensive structured diagnostic interview should be undertaken to confirm the effect of green tea consumption on depressive symptoms. Third, because this study was a cross-sectional study, we could not conclude whether lower green tea consumption increased the

occurrence of depressive symptoms or whether depressive symptoms lead to a decline in green tea consumption. Therefore, a prospective study or trial should be undertaken to confirm the relation between green tea consumption and depressive symptoms. Fourth, we could not make adjustments for a history of depressive disorders, other psychological variables, and associated medications/supplements because data for these were not obtained. However, because all assessments of this study were carried out in a public facility and participation in the study was voluntary, we considered the prevalence of these factors as likely to have been very low, and therefore we believe that not directly accounting for them in our analyses had little effect on the findings. Moreover, although we adjusted for a considerable number of confounding factors, we cannot exclude the possibility that depressive symptoms are affected by the other dietary habits that correlate with the habitual consumption of green tea. Therefore, an intervention study is necessary for establishing a causal relation between green tea consumption and depressive symptoms.

In the present study, higher green tea consumption (as measured by self-administered questionnaires) was significantly associated with a lower prevalence of depressive symptoms in community-dwelling elderly individuals. This finding suggested that the consumption of green tea may have a potentially beneficial effect on the prevention of depressive symptoms. A prospective study or randomized trials are required to clarify the causality.

The authors' responsibilities were as follows—KN and AH: study concept and design; KN, AH, SK, SE, NN, KO-M, HT, YM, HA, SA, RN, and IT: acquisition of subjects and data; KN, AH, SK, SE, HG, NN, KO-M, HT, YM, MA, SS, HA, SA, and RN: analysis and interpretation of data; KN, AH, HG, and MA: preparation of manuscript; SS, HA, SA, RN, and IT: supervision; and IT: obtaining funding. None of the authors had a conflict of interest.

REFERENCES

1. Cronin-Stubbs D, de Leon CF, Beckett LA, Field TS, Glynn RJ, Evans DA. Six-year effect of depressive symptoms on the course of physical disability in community-living older adults. Arch Intern Med 2000;160:3074–80.
2. Ng TP, Niti M, Tan WC, Cao Z, Ong KC, Eng P. Depressive symptoms and chronic obstructive pulmonary disease: effect on mortality, hospital readmission, symptom burden, functional status, and quality of life. Arch Intern Med 2007;167:60–7.
3. Pittenger C, Duman RS. Stress, depression, and neuroplasticity: a convergence of mechanisms. Neuropsychopharmacology 2008;33:88–109.
4. Licinio J, Wong ML. The role of inflammatory mediators in the biology of major depression: central nervous system cytokines modulate the biological substrate of depressive symptoms, regulate stress-responsive systems, and contribute to neurotoxicity and neuroprotection. Mol Psychiatry 1999;4:317–27.
5. Zorrilla EP, Luborsky L, McKay JR, et al. The relationship of depression and stressors to immunological assays: a meta-analytic review. Brain Behav Immun 2001;15:199–226.
6. Balentine DA, Wiseman SA, Bouwens LC. The chemistry of tea flavonoids. Crit Rev Food Sci Nutr 1997;37:693–704.
7. Nathan PJ, Lu K, Gray M, Oliver C. The neuropharmacology of L-theanine(N-ethyl-L-glutamine): a possible neuroprotective and cognitive enhancing agent. J Herb Pharmacother 2006;6:21–30.
8. Kimura K, Ozeki M, Juneja LR, Ohira H. L-Theanine reduces psychological and physiological stress responses. Biol Psychol 2007;74:39–45.
9. Cao H, Kelly MA, Kari F, et al. Green tea increases anti-inflammatory tristetraprolin and decreases pro-inflammatory tumor necrosis factor mRNA levels in rats. J Inflamm (Lond) 2007;4:1.
10. Mahajan N, Dhawan V, Sharma G, Jain S, Kaul D. Induction of inflammatory gene expression by THP-1 macrophages cultured in normocholesterolaemic hypertensive sera and modulatory effects of green tea polyphenols. J Hum Hypertens 2008;22:141–3.
11. Hsu SP, Wu MS, Yang CC, et al. Chronic green tea extract supplementation reduces hemodialysis-enhanced production of hydrogen peroxide and hypochlorous acid, atherosclerotic factors, and proinflammatory cytokines. Am J Clin Nutr 2007;86:1539–47.
12. Tipoe GL, Leung TM, Hung MW, Fung ML. Green tea polyphenols as an anti-oxidant and anti-inflammatory agent for cardiovascular protection. Cardiovasc Hematol Disord Drug Targets 2007;7:135–44.
13. Hozawa A, Kuriyama S, Nakaya N, et al. Inverse relation between green tea consumption and psychological distress as assessed by K6 in Japanese general population: the Ohsaki Cohort 2006 Study. Am J Clin Nutr (in press).
14. Shimbo M, Nakamura K, Jing Shi H, et al. Green tea consumption in everyday life and mental health. Public Health Nutr 2005;8:1300–6.
15. Niu K, Hozawa A, Guo H, et al. Serum C-reactive protein even at very low (<1.0 mg/l) concentration is associated with physical performance in a community-based elderly population aged 70 years and over. Gerontology 2008;54:260–7.
16. Niu K, Hozawa A, Kuriyama S, et al. Dietary long-chain n−3 fatty acids of marine origin and serum C-reactive protein concentrations are associated in a population with a diet rich in marine products. Am J Clin Nutr 2006;84:223–9.
17. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state." A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189–98.
18. Niino N, Imaizumi T, Kawakami N. A Japanese translation of the Geriatric Depression Scale. Clin Gerontol 1991;10:85–7.
19. Science and Technology Agency. [Standard Tables of Food Composition in Japan.] 5th rev. ed. Tokyo, Japan: Printing Bureau, Ministry of Finance, 2000 (in Japanese).
20. Sakai K, Nakajima M, Watanabe S, Kobayashi T. [Available data on assessments of dietary fatty acid intake (1).] J Lipid Nutr 1995;4:97–103 (in Japanese).
21. Sasaki S. Serum biomarker-based validation of a brief-type self-administered diet history questionnaire for Japanese subjects (in Japanese). The Study Group of Ministry of Health, Labor and Welfare of Japan, Tanaka H, chairman. "A research for assessment of nutrition and dietary habit in "Kenko Nippon 21." Tokyo 2005:10–42.
22. Chobanian AV, Bakris GL, Black HR, et al. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. JAMA 2003;289:2560–72.
23. Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 2003;107:499–511.
24. Kuriyama S, Hozawa A, Ohmori K, et al. Green tea consumption and cognitive function: a cross-sectional study from the Tsurugaya Project. Am J Clin Nutr 2006;83:355–61.
25. Muraoka Y, Oiji A, Ihara K. [The physical and psychological and social background factor of elderly depression in the community.] Jpn J Geriatr Psychiatry 1996;7:397–407 (in Japanese).
26. Koyano W, Shibata H, Haga H, Suyama Y, Nakazato K. [Measurement of competence in the elderly living at home: development of an index of competence.] Nippon Koshueiseigaku Zasshi 1987;34:109–14 (in Japanese).
27. Awata S, Seki T, Koizumi Y, et al. Factors associated with suicidal ideation in an elderly urban Japanese population: a community-based, cross-sectional study. Psychiatry Clin Neurosci 2005;59:327–36.
28. Niu K, Hozawa A, Fujita K, et al. Influence of leisure-time physical activity on the relationship between C-reactive protein and hypertension in a community-based elderly population of Japan: the Tsurugaya project. Hypertens Res 2005;28:747–54.
29. Alpert JE, Mischoulon D, Nierenberg AA, Fava M. Nutrition and depression: focus on folate. Nutrition 2000;16:544–6.
30. Beydoun MA, Kuczmarski MT, Mason MA, Ling SM, Evans MK, Zonderman AB. Role of depressive symptoms in explaining

socioeconomic status disparities in dietary quality and central adiposity among US adults: a structural equation modeling approach. Am J Clin Nutr 2009;90:1084–95.
31. Alves de Rezende CH, Coelho LM, Oliveira LM, Penha Silva N. Dependence of the geriatric depression scores on age, nutritional status, and haematologic variables in elderly institutionalized patients. J Nutr Health Aging 2009;13:617–21.
32. Yokogoshi H, Kobayashi M, Mochizuki M, Terashima T. Effect of theanine, r-glutamylethylamide, on brain monoamines and striatal dopamine release in conscious rats. Neurochem Res 1998;23:667–73.
33. Delgado PL. Depression: the case for a monoamine deficiency. J Clin Psychiatry 2000;61(suppl 6):7–11.
34. Bryan J. Psychological effects of dietary components of tea: caffeine and L-theanine. Nutr Rev 2008;66:82–90.
35. Kessler RC, Andrews G, Colpe LJ, et al. Short screening scales to monitor population prevalences and trends in non-specific psychological distress. Psychol Med 2002;32:959–76.

The American Journal of Clinical Nutrition

Peptides 30 (2009) 2316–2322

Contents lists available at ScienceDirect

Peptides

journal homepage: www.elsevier.com/locate/peptides

Regular paper

Gene expression of (pro)renin receptor is upregulated in hearts and kidneys of rats with congestive heart failure

Takuo Hirose [a,*], Nobuyoshi Mori [b], Kazuhito Totsune [a,c], Ryo Morimoto [d], Takahiro Maejima [e], Takuya Kawamura [a], Hirohito Metoki [a,f], Kei Asayama [c], Masahiro Kikuya [a], Takayoshi Ohkubo [c,e], Masahiro Kohzuki [b], Kazuhiro Takahashi [g], Yutaka Imai [a,c]

[a] Department of Clinical Pharmacology and Therapeutics, Tohoku University Graduate School of Pharmaceutical Sciences and Medicine, 6-3 Aramaki-aza-Aoba, Aoba-ku, Sendai 980-8578, Japan
[b] Department of Internal Medicine and Rehabilitation Science, Tohoku University Graduate School of Medicine, 1-1 Seiryo-machi, Aoba-ku, Sendai 980-8574, Japan
[c] Tohoku University 21st Century Center of Excellence Program "Comprehensive Research and Education Center for Planning of Drug Development and Clinical Evaluation (CRESCENDO)", 6-3 Aramaki-aza-aoba, Aoba-ku, Sendai 980-8578, Japan
[d] Division of Nephrology, Endocrinology, and Vascular Medicine, Department of Medicine, Tohoku University Graduate School of Medicine, 1-1 Seiryo-machi, Aoba-ku, Sendai 980-8574, Japan
[e] Department of Planning for Drug Development and Clinical Evaluation, Tohoku University Graduate School of Pharmaceutical Sciences and Medicine, 2-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan
[f] Department of Medical Genetics, Tohoku University Graduate School of Medicine, 1-1 Seiryo-machi, Aoba-ku, Sendai 980-8574, Japan
[g] Department of Endocrinology and Applied Medical Science, Tohoku University Graduate School of Medicine, 2-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan

ARTICLE INFO

Article history:
Received 1 June 2009
Received in revised form 10 September 2009
Accepted 10 September 2009
Available online 16 September 2009

Keywords:
Renin–angiotensin system
Organ damage
RT-PCR
Immunohistochemistry

ABSTRACT

Recent studies have revealed that (pro)renin receptor ((P)RR), a newly identified member of the renin–angiotensin system, was associated with organ damage in the kidney. However, there has been little information for (P)RR in hearts. To investigate the regulation of (P)RR in heart failure, we examined the expression of (P)RR in hearts and kidneys of rats with congestive heart failure (CHF) due to coronary ligation by quantitative RT-PCR and immunohistochemistry. Significantly increased levels of (P)RR mRNA were found in the atrium, right ventricle, non-infarcted part of left ventricle, infarcted part of left ventricle and kidney of CHF rats, when compared with sham operated rats (about 1.6-fold, 1.4-fold, 1.6-fold, 1.7-fold and 1.5-fold, respectively). Expression levels of mRNAs encoding renin and angiotensinogen in these heart and kidney tissues were also increased in the CHF rats. Immunohistochemistry showed positive (P)RR immunostaining in the myocardium, the renal tubular cells, and vascular smooth muscle and endothelial cells in the heart and the kidney. The renal tubular cells were more intensely immunostained in CHF rats than in sham operated rats. These findings suggest that the expression of (P)RR is increased in the hearts and kidneys of rats with heart failure, and that (P)RR may contribute to heart failure.

1. Introduction

The renin–angiotensin system (RAS) plays an essential role in the regulation of blood pressure and electrolyte balance [4,16,31]. The RAS components including renin, angiotensinogen and angiotensin-converting enzyme (ACE) are expressed in the heart and their gene expression is upregulated in the cardiac tissue with heart failure [23,32]. The RAS is also involved in cell growth, fibrosis, and inflammation in cardiovascular and renal tissues as locally produced and locally acting factors [4,16,31], and may play important pathophysiological roles in the cardiac hypertrophy and fibrosis in heart failure.

Recently, (pro)renin receptor ((P)RR), a specific receptor for renin and prorenin, was newly identified as a member of the RAS by Nguyen et al. [21,22,27]. (P)RR is a 350 amino-acid protein with a single transmembrane domain and widely expressed in various tissues including heart, kidney and brain [22]. When bound to (pro)renin, (P)RR leads nonproteolytic activation of prorenin and directly activate mitogen-activated protein kinase (MAPK) ERK1/2 independently from the RAS [8,22]. Several animal studies showed that (P)RR contributed to blood pressure regulation or development of end organ damages [3,9–12,14]. Burcklé et al. reported that elevated blood pressure and heart rate were observed in rats with human (P)RR gene over-expression [3]. Ichihara et al.

* Corresponding author. Tel.: +81 22 795 6807; fax: +81 22 795 6839.
E-mail address: hirose-t@m.tains.tohoku.ac.jp (T. Hirose).

0196-9781/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.peptides.2009.09.015

reported that development of cardiac fibrosis or renal damage in stroke-prone spontaneously hypertensive rats (SHR-sp) was ameliorated by blocking of (P)RR with handle region peptide, a peptide corresponding to the handle region of the prorenin prosegment [10,11]. We have recently reported that polymorphism of the (P)RR gene IVS5+169C>T is associated with ambulatory blood pressure in Japanese men, suggesting that (P)RR has a certain role in blood pressure regulation in men [6].

The pathophysiological role of (P)RR in congestive heart failure (CHF), however, has not been clarified yet. In this study, we therefore examined the expression of (P)RR in hearts and kidneys of CHF rats induced by coronary ligation.

2. Methods

2.1. Animals

Animal experiments were performed in accordance with the NIH guide for the Care and Use of Laboratory Animals and were approved by the Animal Care Committee of Tohoku University.

Eight-week-old male Wistar-Kyoto (WKY) rats (Charles River Japan, Tsukuba, Japan) underwent either coronary ligation or sham operation under sodium pentobarbital anesthesia (50 mg/kg), as previously described [7,20,33]. In short, in the coronary ligation group (CHF rats, $n = 35$), a left thoracotomy was performed and the left coronary artery was ligated between the pulmonary trunk and the left auricle. Sham operated (SO) rats ($n = 6$) were treated similarly, but without the suture around the coronary artery. Rats received standard rat chow and water *ad libitum* for the 8 weeks after surgery.

All rats underwent blood pressure measurement and echocardiography one day before organ collection under anesthesia. Blood samples were obtained during the organ collection and plasma brain natriuretic peptide (BNP) levels were measured by SRL (Tokyo, Japan). Twenty-five out of the 35 rats which underwent the coronary ligation were excluded from the study because of death, mild infarction with infarct size less than 30% or normal ejection fraction on echocardiography. The remaining ten rats were used as CHF rats in this study. In CHF rats, when compared with SO rats, significantly elevated levels of heart weight (about 1.3-fold), lung weight (about 1.6-fold), systolic left ventricular inner diameter (about 1.6-fold), diastolic left ventricular inner diameter (about 1.2-fold) and BNP (about 1.6-fold) and significantly decreased mean arterial blood pressure (about 87%), ejection fraction (about 56%) and fractional shortening (about 48%) were observed (Table 1).

The atria, right ventricles, non-infarcted and the infarcted part of the left ventricles and the kidneys were harvested, snap-frozen in liquid nitrogen and maintained at −80 °C until RNA extraction.

Table 1
Characteristic data for sham operated (SO) rats and congestive heart failure (CHF) rats.

Rats	SO ($n=6$)	CHF ($n=10$)
BW (g)	345 ± 9	343 ± 9
HW (g)	1.18 ± 0.13	1.56 ± 0.09*
LW (g)	1.35 ± 0.04	2.10 ± 0.23*
MAP (mmHg)	107 ± 2	93 ± 3*
HR (bpm)	235 ± 6	240 ± 9
LVIDs (mm)	4.83 ± 0.33	7.82 ± 0.35*
LVIDd (mm)	7.93 ± 0.31	9.54 ± 0.29*
EF (%)	77.7 ± 1.8	43.6 ± 3.1*
FS (%)	37.3 ± 2.0	18.0 ± 1.5*
BNP (pg/ml)	101 ± 12	164 ± 18*

BW, body weight; HW, heart weight; LW, lung weight; MAP, mean arterial blood pressure; HR, heart rate; bpm, beat per minutes; LVIDs, end-systolic left ventricular inner diameter; LVIDd, end-diastolic left ventricular inner diameter; EF, ejection fraction of left ventricle; FS, fractional shortening of left ventricle; BNP, plasma brain natriuretic peptide concentration.
Values are mean + SEM.
* $P < 0.05$ vs. sham operated rats by unpaired Student's *t*-test.

The whole atria were used for the RNA extraction without separation into right and left atrium. In CHF rats, the thin fibrotic infarcted region was carefully dissected from the viable non-ischemic myocardium and used as the infarcted tissue sample. Tissues for histological examination were excised, fixed with 10% neutral buffered formalin and embedded into paraffin.

Furthermore, age matched (16 weeks old) male WKY were killed under anesthesia, and the heart was harvested for Western blot analysis.

2.2. RNA extraction and competitive, quantitative RT-PCR

Total RNA was extracted by the guanidinium isothiocyanate/cesium chloride method, and 4 μg of total RNA were reverse transcribed with 400 units of Moloney Murine Leukemia Virus reverse transcriptase (PrimeScript; TaKaRa, Otsu, Japan) using an oligo(dT) primer, as previously described [7,20,29].

Expression levels of (P)RR, renin and angiotensinogen mRNAs were determined using competitive, quantitative RT-PCR methods. Since expression levels of ribosomal protein L32 (RPL32) mRNA were the most stable among the several housekeeping genes, we used RPL32 mRNA concentrations as internal control [7]. The primers used for the RT-PCR analysis are summarized in Table 2. To determine the equivalent concentration point, the competitive reference standard (CRS-) DNA for (P)RR, renin, angiotensinogen and RPL32 was prepared as reported previously [7,15,20,25,26,29]. The CRS-DNA for (P)RR, renin and angiotensinogen has 765-bp

Table 2
The sequence of the primers for polymerase chain reaction.

Gene	Primer sequence (5′-3′)	Position	PCR products	GenBank accession no.
(Pro)renin receptor				
Sense	CCATGGCTGTGCTTGTCGTTCTC	84–106	958-bp	AB188298
Antisense	AAGGCCAAGCCAGTCATAATCCAC	1018–1041		
Renin				
Sense	CGAGGTGCTAAAGGAGGAAGTG	857–878	551-bp	NM012642
Antisense	ACCCGATGCGATTGTTATGC	1388–1407		
Angiotensinogen				
Sense	CAGCACGGACAGCACCCTATT	983–1003	428-bp	NM134432
Antisense	TGCCCAGATTTGCCTCAGC	1392–1410		
Ribosomal protein L32				
Sense	AAGTTCATCAGGCACCAGTC	110–129	279-bp	NM013226
Antisense	GATGGCTTTTCGGTTCTTAG	369–388		

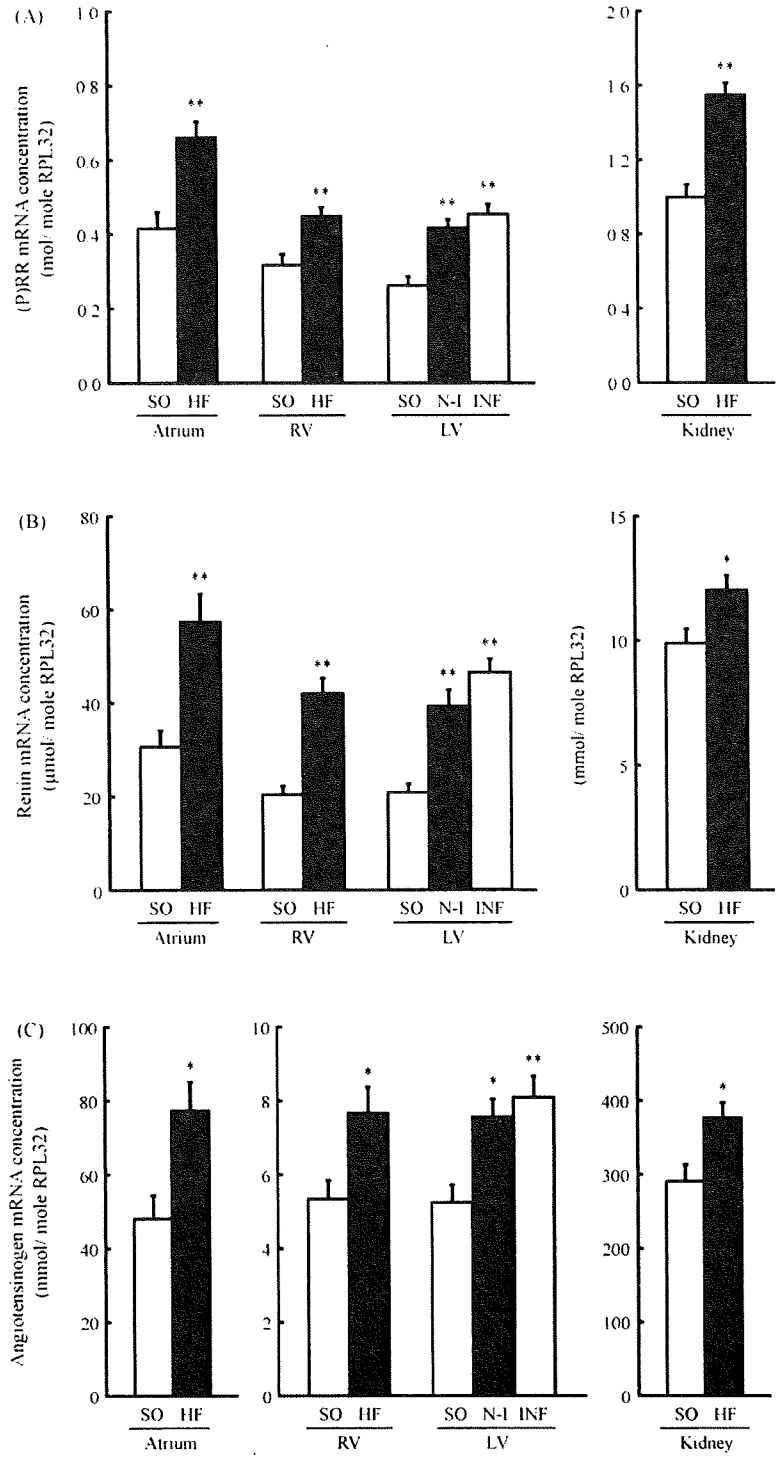

Fig. 1. Expression levels of (pro)renin receptor ((P)RR) mRNA (A), renin mRNA (B) and angiotensinogen mRNA (C) in the atrium, the right ventricle (RV), the left ventricle (LV) and the kidney. These tissues were obtained from sham operated (SO) rats ($n = 6$) and congestive heart failure (CHF) rats ($n = 10$). Values are mean ± SEM. N-I: non-infarcted part of LV obtained from CHF rats. INF: infarcted part of LV obtained from CHF rats. $^{*}P < 0.05$ vs. SO rats, $^{**}P < 0.01$ vs. SO rats.

length (193-bp deletion), 401-bp length (150-bp deletion) and 330-bp length (98-bp deletion), respectively. A constant amount of wild-type cDNA and increasing amounts of CRS-DNA were added to each PCR tube. Every PCR reaction was repeated 3 times, and then the mean value was calculated.

The obtained PCR products were purified by agarose gel, sequenced by an autosequencer (Model 3100; Applied Biosystems, Foster, CA), and confirmed 100% identity with the respective nucleotide sequence registered in the NCBI Data Bases.

2.3. Antiserum

The antiserum against (P)RR was raised in a rabbit by injecting the peptide fragment of human (P)RR corresponding to 224–237 a.a. (human (P)$RR_{224-237}$, Custom synthesis; TaKaRa, Otsu, Japan) conjugated with bovine serum albumin. The amino acid sequence of human (P)$RR_{224-237}$ had 100% identity with that of rat (P)$RR_{223-236}$. The same rabbit serum before the injection of the antigen peptide (non-immune rabbit serum) was used as a negative control.

2.4. Immunohistochemistry

Immunohistochemistry of (P)RR was performed by the ABC method using the Vector ABC kit (Vector Laboratories, Burlingame, CA), as previously reported [7,18,28]. Briefly, 1.5 μm sections were deparaffinized and incubated in methanol containing 0.3% H_2O_2 for 30 min and then with normal goat serum (1:20) to block non-specific staining. Sections were intensely washed in PBS between the procedures. The sections were then incubated in antiserum against (P)RR (1:500) or non-immune rabbit serum (1:500) (the negative control) for 20 h at 4 °C. Sections were incubated in biotinylated secondary antibody to rabbit IgG (1:400) for 30 min at room temperature and subsequently incubated with peroxidase-conjugated avidin for 30 min using the Vector ABC kit. These sections were visualized by immersion in 3,3′-diaminobenzidine solution (0.01 mol/l 3,3′-diaminobenzidine in 0.05 mol/l Tris–HCl buffer (pH 7.6) and 0.006% H_2O_2).

The specificity of the (P)RR antiserum was examined by the absorption test. The diluted antiserum (1:500) was incubated with the antigen peptide at concentrations of 10 nmol peptide/ml of the diluted antiserum for 20 h at 4 °C prior to use.

2.5. Western blot analysis

(P)RR-immunoreactivity in the rat heart was characterized by Western blot analysis. Western blot analysis was performed as previously described [13]. In brief, the heart tissues of 16-week-old male WKY were homogenized in 3 ml of a 10 mmol/l potassium buffer (pH 7.7) containing 250 mmol/l sucrose, 1 mmol/l EDTA and 0.1 mmol/l phenylmethylsulfonyl fluoride. The homogenates were centrifuged at 3000 × *g* for 5 min and 9000 × *g* for 15 min. The supernatants were separated by electrophoresis on a 12% sodium dodecyl sulfate polyacrylamide gel for 2 h at 150 V and transferred electrophoretically to a nitrocellulose membrane at 100 V in a transfer buffer consisting of 25 mmol/l Tris–HCl, 192 mmol/l glycine and 20% methanol for 1 h at 4 °C. The membranes were blocked overnight at 4 °C by immersion into a Tris-borate-saline Tween-20 (TBST-20) buffer (10 mmol/l Tris–HCl, 150 mmol/l NaCl, and 0.08% Tween-20) containing 10% nonfat dry milk. The membrane was incubated for 1 h with antiserum against (P)RR (1:4000), or antiserum against (P)RR (1:4000) which was incubated with the antigen peptide for 20 h at 4 °C, prior to use. The membrane was washed several times with TBST-20 buffer and then incubated with horseradish peroxidase-coupled secondary antibody (1:20000; Santa Cruz Biotechnology, Santa Cruz, CA) for 1 h. The membrane was washed with TBST-20 buffer and then developed using an enhanced chemiluminescence kit (ECL Western blotting detection system; Amersham, Arlington Heights, IL).

2.6. Statistical analysis

Data are given as mean ± SEM. mRNA concentrations were analyzed by unpaired Student's *t*-test or one-way analysis of variance (ANOVA) and Scheffe's post hoc test for multiple comparison of differences among the groups. A P value of less than 0.05 was considered statistically significant.

3. Results

3.1. mRNA expression

In the CHF rats, (P)RR mRNA levels were significantly increased by about 1.6-fold in the atrium ($P = 0.002$), 1.4-fold in the right ventricle ($P = 0.004$), 1.6-fold in the non-infarcted part of the left ventricle ($P = 0.003$), 1.7-fold in the infarcted part of the left ventricle ($P < 0.001$) and 1.5-fold in the kidney ($P < 0.001$), when compared with SO rats (Fig. 1A). There was no significant difference in the expression levels of (P)RR mRNA between the non-infarcted part of the left ventricle and the infarcted part of the left ventricle ($P = 0.6$).

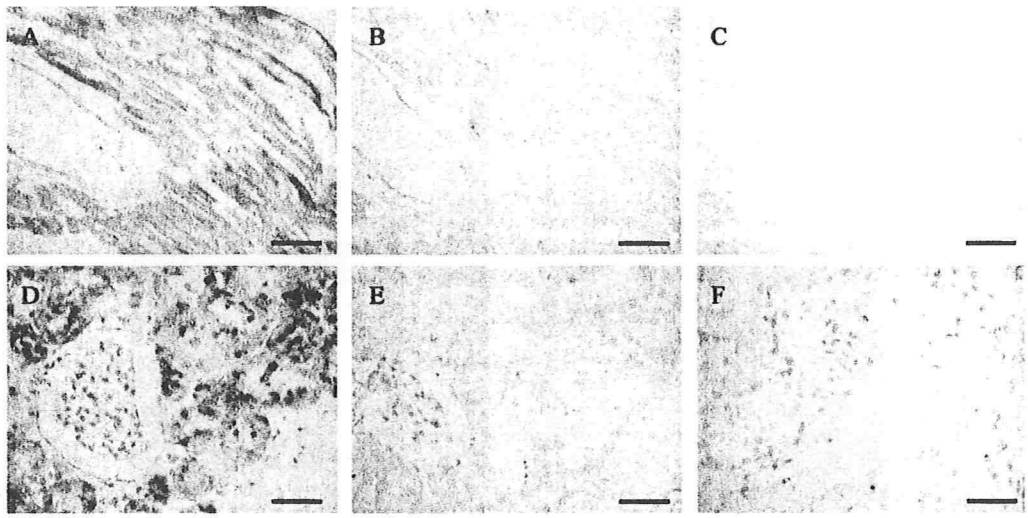

Fig. 2. The absorption test of (pro)renin receptor ((P)RR) antiserum in rat heart ((A)–(C)) and kidney ((D)–(F)). ((A) and (D)) positive control immunostained with (P)RR antiserum in heart (A) and kidney (D). ((B) and (E)) absorption test using (P)RR antiserum preabsorbed with synthetic human (P)$RR_{224-237}$ (10 nmol peptide/ml) in heart (A) and kidney (E). ((C) and (F)) negative control using non-immune rabbit serum in heart (C) and kidney (F). Bar = 50 μm.

The CHF rats showed significantly increased renin mRNA levels by about 1.9-fold in the atrium ($P = 0.001$), 2.1-fold in the right ventricle ($P < 0.001$), 1.8-fold in the non-infarcted part of the left ventricle ($P = 0.02$), 2.0-fold in the infarcted part of the left ventricle ($P = 0.001$) and 1.3-fold in the kidney ($P = 0.002$), when compared with SO rats (Fig. 1B). There was no significant difference in the expression levels of renin mRNA between the non-infarcted part of the left ventricle and the infarcted part of the left ventricle ($P = 0.4$).

Furthermore, the CHF rats showed significantly increased angiotensinogen mRNA levels by about 1.6-fold in the atrium ($P = 0.02$), 1.4-fold in the right ventricle ($P = 0.04$), 1.4-fold in the non-infarcted part of the left ventricle ($P = 0.03$), 1.5-fold in the infarcted part of the left ventricle ($P = 0.007$) and 1.3-fold in the kidney ($P = 0.02$), when compared with SO rats (Fig. 1C). There was no significant difference in the expression levels of angiotensinogen mRNA between the non-infarcted part of the left ventricle and the infarcted part of the left ventricle ($P = 0.8$).

3.2. Immunohistochemistry

Immunohistochemistry of (P)RR in rat hearts and kidneys showed positive immunostaining in normal rat heart (Fig. 2A) and kidney (Fig. 2D). The absorption of the antiserum by the antigen peptide attenuated positive immunostaining significantly in normal rat heart (Fig. 2B) and kidney (Fig. 2E). The negative control using non-immune rabbit serum showed no positive immunostaining (Fig. 2C and F).

The myocardium was diffusely immunostained with (P)RR in SO rats (Fig. 3A, C, and E) and CHF rats (Fig. 3B, D, and F). The vascular smooth muscle and endothelial cells in the heart were positively immunostained for (P)RR in both SO rats (Fig. 3E) and CHF rats (Fig. 3F). The infarcted area was not immunostained with

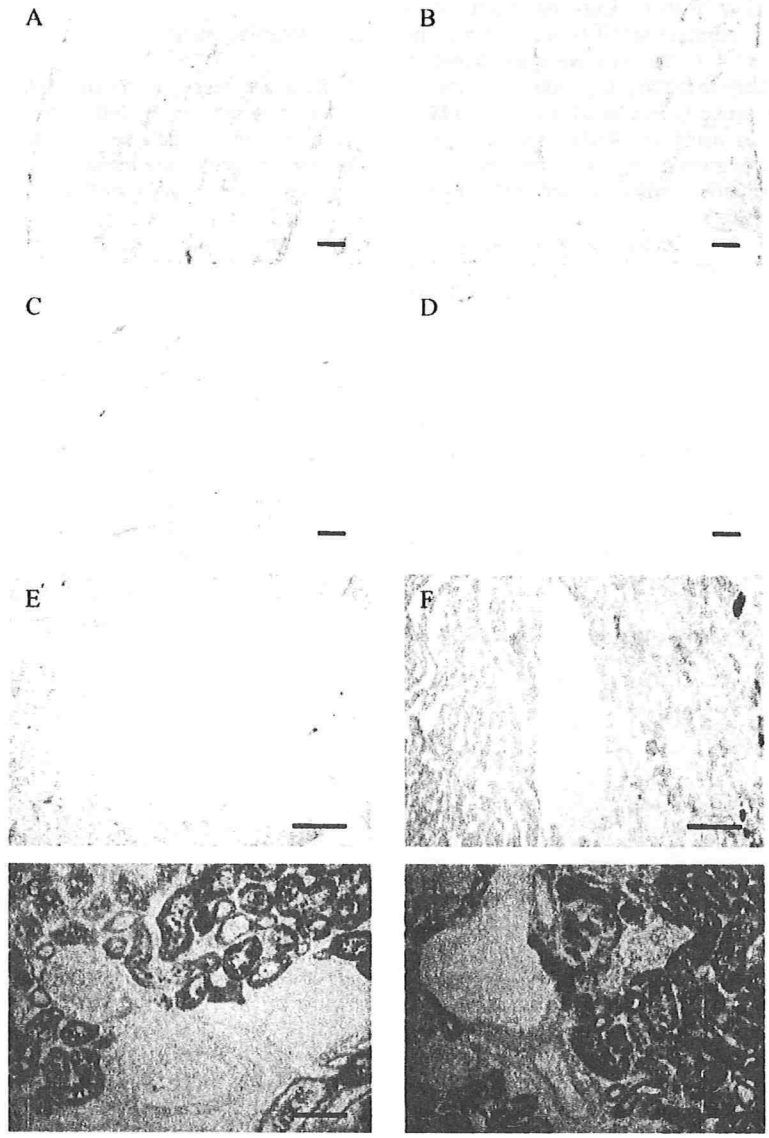

Fig. 3. Immunohistochemistry of (pro)renin receptor in rat heart ((A)–(F)) and kidney ((G) and (H)). All panels represent at least three samples. ((A) and (B)) the right ventricle of sham operated (SO) rats (A) and congestive heart failure (CHF) rats (B). ((C) and (D)) the left ventricle of SO rats (C) and CHF rats (D). ((E) and (F)) the blood vessels of SO rats (E) and CHF rats (F). ((G) and (H)) the kidney of SO rats (G) and CHF rats (H). Bar = 100 μm.

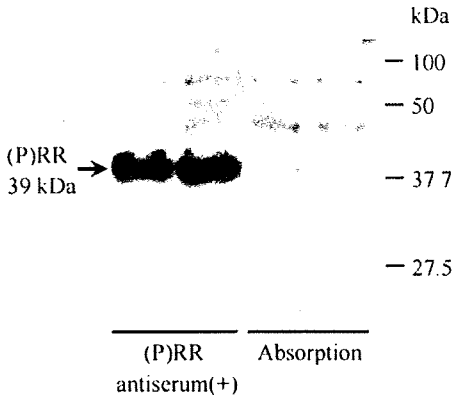

Fig. 4. Western blot of (pro)renin receptor ((P)RR) in the heart. A single signal corresponding to a 39 kDa was detected when (P)RR antiserum was used. The signal of (P)RR was hardly detectable when (P)RR antiserum preabsorbed with synthetic human $(P)RR_{224-237}$ was used instead of the (P)RR antiserum.

(P)RR antiserum except scattered cardiomyocytes (Fig. 3D). In the kidney, renal tubular cells were diffusely immunostained for both SO rats (Fig. 3G) and CHF rats (Fig. 3H). The vascular smooth muscle and endothelial cells in the kidney were positively immunostained in both SO rats (Fig. 3G) and CHF rats (Fig. 3H). Particularly strong immunostaining of (P)RR was observed in the renal tubules of CHF rats (Fig. 3G). The glomeruli was sporadically immunostained in both SO rats (Fig. 3G) and CHF rats (Fig. 3H).

3.3. Western blot analysis

The identity of the (P)RR-immunoreactivity in the rat heart was confirmed by Western blot analysis. Western blot analysis of the heart tissue extract showed one band at the expected molecular weight of 39 kDa (Fig. 4). This band of 39 kDa was abolished by preabsorption with the antigen peptide.

4. Discussion

The present study has shown for the first time the increased gene expression of (P)RR in the hearts and kidneys of CHF rats. Although increased gene expression of (P)RR has been reported in the hearts and kidneys in SHR-sp [10,11], there has been no previous report on (P)RR expression in the heart and kidney tissues of the CHF animal model. We have also confirmed the increased cardiac and renal expression of renin and angiotensinogen mRNAs, which is consistent with previous reports [23,24,32]. The prorenin binding to (P)RR has angiotensin I-generating activity in the absence of cleavage of the prosegment [2,19,22,27], and the (P)RR binding renin and prorenin directly activate the MAPK ERK1/2 [8,22,27]. Taken together, increased expression of the (P)RR in the hearts with CHF rats may contribute to cardiac tissue RAS activation and end-organ damage.

Ligation of the left coronary artery in rats induces myocardial hypertrophy by compensatory adaptation [5]. The increased heart weight was found in the CHF rats also in the present study, as shown in Table 1. In the hearts, positive immunostaining of (P)RR were observed in the myocardium and the vascular smooth muscle and endothelial cells of both groups of CHF rats and SO rats. In addition, increased expression levels of (P)RR mRNA in ventricle of CHF rats have been shown by quantitative RT-PCR. The similar expression levels of (P)RR mRNA between the non-infarcted part and the infarcted part of the left ventricle may be due to the high expression levels of (P)RR in scattered cardiomyocytes remaining in the infarcted part. (P)RR expressed in the CHF heart may be related to the cardiac hypertrophy observed in CHF via the direct stimulation of the MAPK signaling in cardiomyocytes and/or the angiotensin II generation.

In the CHF rat kidneys, (P)RR, renin and angiotensinogen mRNA levels were significantly elevated, when compared with SO rats. The hemodynamic consequences of heart failure may elevate the expression levels of (P)RR mRNA in the kidney. It is known that CHF is accompanied by decreased renal circulation, increased renin secretion, and the increased activity of RAS. Increased aldosterone secretion results in increased retention of water and NaCl in CHF. The present findings suggest that upregulated (P)RR expression in the kidney has a certain role in the development of heart failure after myocardial infarction via the circulating RAS activation, and is another cause for the enhanced renal tissue RAS in CHF.

In the kidneys, positive immunostaining of (P)RR were observed in the renal tubular cells and the vascular smooth muscle and endothelial cells of both groups of CHF rats and SO rats. The renal tubular cells were more intensely immunostained in CHF rats than in SO rats. Therefore, our results of immunohistochemistry in the kidney have raised the possibility that (P)RR may act a regulator of the water and electrolyte transport in renal tubular cells via renal RAS activation. This role of (P)RR may be particularly important in the CHF kidney, because of the overload of the water and electrolyte transport for the renal tubular cells resulting in congestion. In this regard, Advani et al. have recently reported that (P)RR is predominantly expressed in collecting ducts and in the distal nephron of the kidney, and may function primarily in distal nephron H^+ transport [1].

Increased expression of (P)RR may therefore be related to the organ damage of heart and kidney in CHF via the activation of tissue RAS and the direct stimulation of the MAPK signaling. It remains to be determined by which pathway the (P)RR contributed more greatly to the organ damage of heart and kidney in CHF. In the previous studies by Ichihara et al., development of cardiac fibrosis or renal damage in SHR-sp was ameliorated by blocking of (P)RR with handle region peptide and it was more effective than the ACE inhibitor [10,11]. The present study has raised the possibility that blocking of (P)RR may be a novel strategy against the organ damage of the hearts and kidneys in CHF.

Véniant et al. reported that a 400-fold increase in circulating prorenin exhibited severe cardiac remodeling and renal lesions in the absence of hypertension in transgenic rats expressing prorenin exclusively in the liver [30]. In contrast, Mercure et al. have recently shown that chronic increases in circulating native prorenin (13- to 66-fold) did not result in an increase in cardiac fibrosis or renal glomerular injury in prorenin transgenic mice [17]. These mice showed mild hypertension, which could be abolished by the inhibition of the ACE by captopril but not by the application of HRP, indicating that this blood pressure effect was exclusively due to angiotensin II but not to the (P)RR. Thus, the pathological significance of (P)RR and elevated circulating prorenin in cardiac fibrosis or renal glomerular injury is still controversial. On the other hand, it is noteworthy that tissue renin mRNA expression levels are increased in the heart and kidney tissues of the CHF rats in the present study, raising the possibility that increased tissue levels of locally produced prorenin, together with upregulated (P)RR, may be related to the cardiac fibrosis or renal glomerular injury in heart failure.

The present study has shown that gene expression of (P)RR was elevated in the hearts and kidneys of CHF rats. These findings raise the possibility that (P)RR contributes to myocardial infarction and the heart failure syndrome.

Conflict of interest

The authors declared no conflict of interest.