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## Abstract

### Reversible Cognitive Impairment after Pioglitazone Treatment of an Elderly Woman with Type 2 Diabetes Mellitus and Alzheimer's Disease

Toshioki Matsuzawa, Takashi Sakurai, Taichi Akisaki, Hiroshi Yoshino, Toshihiro Takata, and Koichi Yokono

Department of Internal and Geriatric Medicine, Kobe University Graduate School of Medicine

A 81-year-old woman admitted for memory disturbance and type 2 diabetes mellitus was obese and hypertensive. Serum HbA1c was 8.7% and HOMA-R 5.08. Her standard score of mini mental state examination (MMSE) was 24 and 21 in Hasegawa's dementia scale-revised (HDS-R). She showed serious impairment in delayed recall, orientation, and executive function, and was diagnosed with probable Alzheimer's disease (AD) based on diagnostic criteria of the National Institute of Neurological and Communicative Disorders and Strokes-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA). She was treated with metformin and glimepiride for diabetes and donepezil for AD, but her brain function declined to 19 in MMSE and 17 in HDS-R after 18 months. We added pioglitazone, and after 5 months, her HbA1c fell to 6.3% and her HOMA-R to 2.89. Her brain dysfunction improved in several cognitive domains including short-term memory. Her MMSE score recovered to 24 and her HDS-R to 21, and she showed several improvements in daily performance. Pioglitazone thus appears to partially restore the cognitive AD decline in elderly diabetic patients.

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# アルツハイマー病

## —基礎研究から予防・治療の新しいパラダイム—

III. 臨　床　編

アルツハイマー病の新しい治療法の開発

新規医薬品

## チアゾリジン誘導体

櫻井　孝　　横野浩一

III. 臨 床 編

アルツハイマー病の新しい治療法の開発
新規医薬品

# チアゾリジン誘導体

Thiazolidine derivatives

櫻井 孝　横野浩一

Key words：チアゾリジン誘導体，アルツハイマー病，糖尿病

## はじめに

チアゾリジン誘導体(TZD)はインスリン抵抗性改善薬であり，世界的にはpioglitazone(Actos)とrosiglitazone(Avandil)の2種類が発売されており，我が国では前者のみが承認されている．TZDはブドウ糖・脂質代謝を改善するのみではなく，抗炎症作用，抗動脈硬化作用が報告されており，最近ではアルツハイマー病(AD)の治療薬としても期待されている．一方，TZDには副作用として，浮腫，体重増加，心不全があり，その使用にあたっては慎重な管理を要する．今日，我が国は超高齢社会にあり，後期高齢者になって寝たきりとなる原病をたどるとその多くは生活習慣病である．サクセスフルエイジングのためにもTZDを有効に利用し，生活習慣病や認知症の進展を抑制することが重要である．

本稿では特にAD治療薬としてのTZDについて，最近の知見をまとめ紹介したい．

## 1. チアゾリジンの作用機序

ペルオキシゾーム増殖因子活性化受容体(peroxisome proliferators-activated receptor: PPAR)は，核内受容体スーパーファミリーの一つで，体内および食品に存在する低分子量の脂溶性生理活性物質(15-deoxy $\Delta^{12,14}$ prostaglandin J2など)をリガンドとしている．PPARsは主に糖・脂質代謝にかかわる遺伝子群の発現制御を行い，PPARには$\alpha$，$\beta/\delta$，$\gamma$の3つのタイプが同定されている．PPAR$\alpha$アゴニストであるフィブラート系薬剤は高脂血症治療薬として，PPAR$\gamma$アゴニストであるTZDは糖尿病治療薬として臨床応用されている．PPAR$\gamma$には更に2つのアイソフォームがあり，脂肪組織にPPAR$\gamma$2が，マクロファージおよび血管にPPAR$\gamma$1が存在する．PPAR$\gamma$2は脂肪細胞の分化を促進し，糖取り込みやインスリン感受性にかかわる分子の発現を亢進させる．また同時にアディポネクチンの発現を亢進させる．PPAR$\gamma$1はNF-$\kappa$Bとダイマーを形成し，その作用を阻害することで，MCP-1，VACM-1，TNF-$\alpha$，COX-2，CRPなどの炎症性マーカーを抑制する[1]．

脳ではPPAR$\gamma$は，神経細胞，グリア細胞および脳血管に存在する．TZDはPPAR$\gamma$活性化により炎症物質の発現を抑制する．神経変性疾患，虚血を問わず，炎症は神経細胞死を誘導する因子であり，このためPPAR$\gamma$はADのみならず，グルタミン酸による神経障害，脳虚血，パ

Takashi Sakurai, Koichi Yokono：Department of Geriatric Medicine, Kobe University Graduate School of Medicine　神戸大学大学院医学系研究科 老年内科

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ーキンソン病，多発性硬化症などの疾患でも，治療の新たなターゲットとして期待されている．

## 2. 糖尿病とアルツハイマー病

糖尿病では非糖尿病に比して認知機能障害が認められる．Strachanらの総説では，注意-集中力の低下，前頭葉-遂行機能の障害，視覚性記憶また言語性の記憶低下，精神運動性知能の低下，一般的な知能検査の成績が低下している[2]．しかし糖尿病にみられる認知機能低下は，日常生活に支障を来す程度のものではないと考えられ，これまで多くの関心を集めることはなかった．

ところが近年の疫学調査により，高齢者糖尿病では認知症との合併が多いことが明らかになってきた．糖尿病におけるAD，血管性認知症の相対危険度は，各々1.3-2.3倍，2.0-3.5倍程度とされる[3]．最も信頼性の高い研究とされるRotterdam研究では，インスリン使用者で認知症の相対危険度が4.3倍と高いことが報告された[4]．Honolulu-Asia研究では，ADの遺伝的危険因子であるApo E ε4を保持する高齢者2型糖尿病で相対危険度は更に高いこと，また海馬に老人斑，神経原線維変化が出現すると相対危険度が2.5-3倍高値であることが示され，これまでの臨床解析を裏づける病理成績が示された[5]．現在，どのような高齢者糖尿病で認知機能が低下し，認知症の発症が多いかについて，世界中で研究が進められている．糖尿病の血管性，代謝性要因の中でも，高インスリン血症はADの発症機構の根幹にかかわる可能性があり，以下に述べたい．

## 3. アルツハイマー病における高インスリン血症の関与

インスリンは，脳血管関門を通過し，海馬，大脳皮質，視床下部などに分布するインスリン受容体に結合する．脳内でもインスリンは少量産生される．高インスリン血症では，脳のインスリン取り込みがdown-regulationを受け，長期的には脳内のインスリンシグナルが低下する可能性が提唱されている[6,7]．実際，ADではインスリン受容体が増加しており，インスリン受容体以降のシグナルであるチロシンキナーゼ活性が低下している．

脳においてインスリンは糖エネルギー代謝を調整するばかりではなく，アセチルコリンやノルエピネフリンなどの神経伝達物質の合成を調節し，シナプスの可塑性，記憶や学習に深くかかわる[7]．

一方，インスリンはアミロイドβやタウの代謝にも作用する．インスリンは神経細胞内のアミロイドβの細胞外への分泌を刺激し，またアミロイドβの消化酵素の一つであるインスリン分解酵素(insulin degrading enzyme: IDE)の発現を調整している．ADでは海馬でのIDEの発現が低下しており[8]，IDE関連遺伝子の異常も指摘されている．このため脳内インスリン作用が低下すると，アミロイドβの分解が低下し，アミロイドβの神経細胞内での蓄積が促進される[7]．また，脳から末梢循環中に排出されたアミロイドβのクリアランスが，高インスリン血症により低下する可能性も指摘されている．

更に高インスリン血症では炎症が惹起され，脳脊髄液中のIL-1β，IL-6，TNF-αが増加していることが報告されている[7]．これらの作用を介して高インスリン血症は脳機能を低下させ，AD発症のリスクになると考えられている(図1)．最近ではADにおける脳のインスリン作用不足に伴う代謝異常を3型糖尿病と呼ぶ論文もみられる[9]．

## 4. アルツハイマー病のチアゾリジンによる治療

ADの予防に消炎鎮痛薬(NSAIDs)が有効である可能性が以前より指摘されていたが，NSAIDsのターゲットがPPARγの活性化にあることが明らかとなった[10]．この発見を契機に，また上述のADと高インスリンの関連から，TZDがAD治療に有効であることが期待されている．TZDが炎症性サイトカインを抑制し，アミロイド前駆体蛋白の代謝酵素であるBACE1の発現を軽減させること，アミロイドβの脳内でのクリアランスを亢進させること，またADモデル

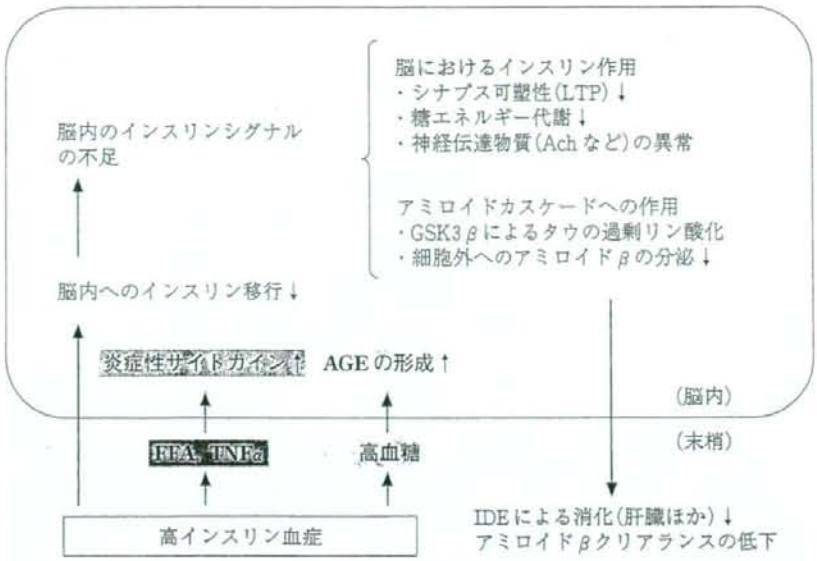


図1 高インスリン血症とアルツハイマー病

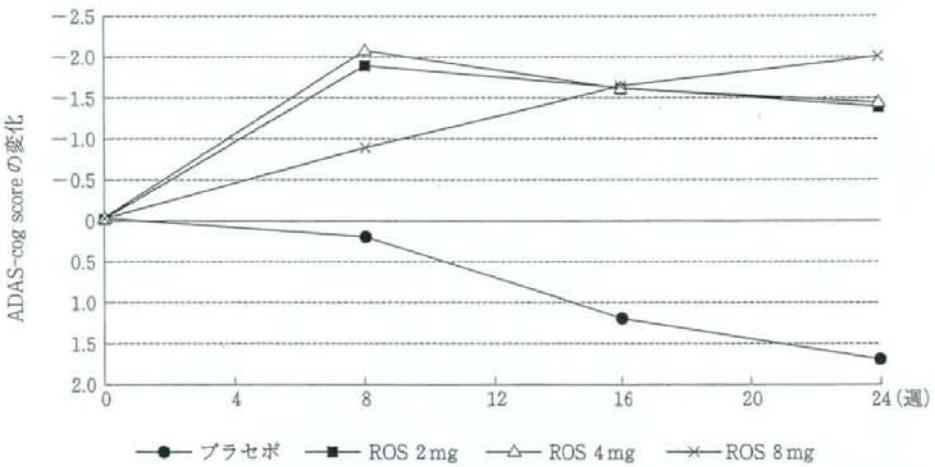


図2 アルツハイマー病に対するロシグリタゾンの効果
Apo E ε4非保持者のみ表示.(文献[11]より改変)

動物で経口投与されたTZDがアミロイドβの蓄積を減少させるなど,基礎的データが蓄積されつつある[10].

最近,軽症のADやamnestic MCIを対象として,rosiglitazoneとプラセボの無作為二重盲検試験が報告された[11].その結果,全体解析では両群間に差はみられなかったが,Apo E ε4非保持者ではrosiglitazone投与群で,記憶や注意力が改善していた(図2).Apo E ε4非保持者のADでは,脳脊髄液/血液インスリン比が低値であるという[7].またrosiglitazone,pioglitazoneは,少量であるが脳へ移行するとの報告もみられ[12],TZDは高インスリン血症改善による作用,抗炎症作用または直接作用により脳機能を改善したものと考えられる.現在,TZDはADのモデュレーターとして治療の選択肢に考えられつ

つあり，米国ではrosiglitazoneを用いた第三相臨床試験が進行している．

## 5. 投与上の注意とまとめ

TZDの副作用として浮腫は重要であり，心不全を惹起するとの報告もある．2003年にはAHAとADAによる共同声明が[13]，2007年にはrosiglitazoneによる心筋梗塞リスクが記載された[14]．ここでは我が国におけるpioglitazoneの市販後調査であるPRACTICAL(糖尿病を対象)の結果を基に説明する[15]．

TZDが浮腫を来す機序として，PPARγ刺激を介した腎尿細管ナトリウム再吸収の促進作用が知られている．浮腫の発現頻度は男性4.2%，女性で12.1%であり，女性に多く，用量依存性に増加する．浮腫のリスクとして，①女性，②糖尿病合併症あり，③糖尿病罹病期間が長い，④高血圧の合併，⑤BMI高値，⑥高齢者があげられる．一方，心不全に進行するリスク要因としては，心不全・心筋梗塞・冠動脈疾患の既往，高血圧，左室肥大，弁膜症，高齢者，糖尿病罹病歴10年以上，インスリンの併用，慢性腎疾患(クレアチニン2.0mg/dl以上)などがある．よって，基本姿勢として投与前後に胸部X線，心エコー，BNP値で心不全・体液貯留をチェックすることが重要である．集合尿細管におけるナトリウムチャネル阻害薬であるトリアムテレン(カリウム保持性利尿薬)が浮腫に有効と考えられるが，実際にはループ利尿薬の使用による浮腫のコントロールが一般的である．

一方，pioglitazoneは心血管障害・脳卒中の予防に有効であることが既に証明されている(PROactive)．同じPPARγ作動薬でありながらrosiglitazoneとpioglitazoneとの差異については不明な点も残るが，現状では慎重な投与が望まれる．

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Cough

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# Cough reflex and oral chemesthesis induced by capsaicin and capsiate in healthy never-smokers

Miyako Yamasaki, Satoru Ebihara*, Takae Ebihara, Shannon Freeman, Shinsuke Yamanda, Masanori Asada, Motoki Yoshida and Hiroyuki Arai

Address: the Department of Geriatrics and Gerontology, Tohoku University School of Medicine, Seiryo-cho 1-1, Aoba-ku, Sendai, 980-8574, Japan

Email: Miyako Yamasaki - ymsk@geriat.med.tohoku.ac.jp; Satoru Ebihara* - s_ebihara@geriat.med.tohoku.ac.jp; Takae Ebihara - takae_montreal@hotmail.com; Shannon Freeman - shannon2@yahoo.ca; Shinsuke Yamanda - fullback15@mtj.biglobe.ne.jp; Masanori Asada - asada@geriat.med.tohoku.ac.jp; Motoki Yoshida - m-yoshida@geriat.med.tohoku.ac.jp; Hiroyuki Arai - satoru_montreal@hotmail.com

* Corresponding author

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## Abstract

**Background:** Many tussive agents are components of foods, but little is known about the relationship between cough reflex and oral chemesthesis sensitivities. We investigated the relationships between cough reflex and oral chemesthesis in individuals using two transient receptor potential vanilloid 1 (TRPV1) agonists with different potencies: capsaicin and capsiate.

**Methods:** Twenty-eight healthy never-smokers were allocated to evaluate cough and oral chemesthesis of capsinoids. Cough reflex sensitivities are estimated by the lowest concentrations generating five coughs by each TRPV1 agonist inhalation. Oral chemesthesis sensitivities are estimated by the lowest concentrations which generate a hot sensation when filter paper loaded with each TRPV1 agonist is placed on the tongue.

**Results:** There were strong correlations between capsaicin- and capsiate-induced cough reflex sensitivities, and between capsaicin- and capsiate-induced oral chemesthesis sensitivities. However, there were no significant correlations between cough reflex and oral chemesthesis sensitivities induced by both capsaicin and capsiate. The cough reflex sensitivities are significantly greater in females than in males whereas there were no gender differences in oral chemesthesis.

**Conclusion:** The results showed that the sensitivities of sensory afferents were different between cough reflex and oral chemesthesis, suggesting that TRPV1 sensitivities differ between organs within healthy individuals. Capsiate could be a tussigen for the cough challenge test.

## Background

Although many tussive agents, such as capsaicin, citric acid, and acetic acid, are components of foods, it is unknown whether these chemical stimuli equally stimulate sensory nerves in bronchial airways and the oral cavity. The inhalation of tussive agents as a cough challenge test is a useful method to quantify cough in a clinical setting and to assess the antitussive effects of specific therapies in a laboratory setting [1]. The inhalation cough challenge is applied via the oral cavity, but little attention has been paid to the effects of tussive agents on oral sensory systems during the cough challenge test. Although,

while testing and developing the inhalation cough challenges, a large number of tussive agents have been tried, capsaicin has stood the test of time and nowadays is the most widely used probably as a result of greater reproducibility and safety [1]. In contrast to classical tastes such as sweet, salty, bitter, sour and umami, the oral sensation induced by capsaicin is called chemesthesis, a sensation of irritation produced by chemical stimulation and mediated by the trigeminal nerve [2].

The physiological effects of capsaicin on cough may be modulated by oral sensory stimuli. Activation of capsaicin-sensitive afferents in the tongue and palate evoke local release of neuropeptides such as substance P and calcitonin gene-related peptides, which are contained in the nerve terminal of the sensory neurons [3,4]. The neuropeptides exert powerful vasoactive and secretomoter effects leading to vasodilation, plasma exudation, triggering reflex salivation and an increase in the secretion of mucus in the airway. Capsaicin is a potent gustatory stimulus which may also promote airway secretions. Gustatory rhinorrhoea has been shown to occur after eating spicy foods and this observation demonstrates a link between gustation and airway secretion of mucus [5]. There is also a possibility that capsaicin in the oral cavity induces bronchoconstriction the same as intranasal application of capsaicin elicits bronchoconstriction [6].

Moreover, in the brain, the gustatory fibers and the sensory fibers that initiate cough may interact with each other because of the close anatomical relationship [7]. In order to inquire into the possible modulation of cough reflex by capsinoid-induced oral stimuli, it might be important to know whether there is a relationship between cough reflex and oral sensitivities to capsinoids. In addition, for the same purpose, it may also be important to know whether there is a gender difference in oral sensitivities to capsinoids since cough reflex sensitivity to capsaicin shows prominent gender differences [8,9].

Capsaicin acts mainly on the afferent neurons of the non-myelinated C-fibers by the opening of a non-selective cation channel of capsaicin receptor, transient receptor potential vanilloid 1 (TRPV1) [10]. Capsiate is obtained from faint-pungent cultivar of red peppers named CH-19 Sweet [11]. CH-19 Sweet is a fixed cultivar that was selected and cultivated from a pungent cultivar, CH-19, of pepper. Capsiate is known to activate TRPV1 [12], and, despite faint-pungency, increases adrenaline secretion and oxygen consumption like capsaicin [13]. *Capsium* fruits are used worldwide in foods for their pungency. The pungency felt when eating *Capsium* fruits is mainly attributed to the activation of oral TRPV1 [14].

TRPV1 receptors found on sensory airway nerves are important in the cough reflex [15]. Isolated pulmonary vagal afferent nerves are responsive to TRPV1 stimulation. When one eats foods containing capsaicin, the burning sensation is elicited by TRPV1-containing peptidergic nociceptors surrounding taste buds in the tongue [16].

Capsaicin-induced cough may not solely be mediated through the nerves expressing TRPV1 receptors. Capsaicin inhalation elicits cough through the activation of rapidly adapting receptors (PAR) [17,18]. The activation of PAR is presumably secondary to airway smooth muscle contraction, mucous secretion or edema formation by capsaicin [18]. Therefore, cough induced by capsaicin is a mixture of direct and indirect responses to the capsaicin. The same situations are also proposed for oral chemesthesis. Despite the complexities of the neural network and involved mechanisms to induce cough or oral chemesthesis, the outcome measurements are relatively simple in these phenomena.

In order to investigate the possible relationship between the perception of sensations mediated by TRPV1, whether directly or indirectly, in different organs, e.g. lung and tongue within individuals, we compared cough reflex and oral chemesthesis sensitivities using two TRPV1 agonists with differential potencies, capsaicin and capsiate. In addition, we evaluated the possibility of the use of capsiate as a cough challenge test.

## Methods

### *Subjects and protocols*

Twenty-eight healthy never-smokers (14 male, 14 female) were allocated to evaluate cough and oral chemesthesis of capsinoids. All were originally recruited via public postings in and around the Tohoku University School of Medicine campus. The mean age was 36.4 ± 2.3 (SE) years. The study was approved by the Institutional Review Boards of Tohoku University School of Medicine. Subjects were without history of pulmonary disease, recent (within 4 weeks) suggestive symptoms, respiratory tract infection and seasonal allergies. Subjects did not take any regular medication.

Subjects underwent the sensitivity tests on four successive days at 10:00 am. Each of the four days was assigned to the capsaicin cough sensitivity test, the capsaicin oral chemethesis test, the capsiate cough sensitivity test, or the capsiate oral chemesthesis test. The order of the four tests was randomly decided using a computer program. The day before the start of the test and during the four days, subjects were prohibited from taking any capsinoids in meals or beverages. In order to ensure subjects avoid consumption of capsinoids during meals, various foods and dishes that contain them were explained to the subjects.

***Cough reflex sensivity tests for capsaicin and capsiate***

Cough reflex sensitivities to capsaicin and capsiate were measured on different days using the modification of the method by Fujimura and colleagues [8]. 30.5 mg of Capsaicin (Sigma Aldrich, Seatle, USA) was dissolved in Tween 80 (1 ml) and ethanol (1 ml) and then dissolved in physiological saline (8 ml) to make a stock solution of 0.01 M, which was stored at -20°C. This solution was diluted with physiological saline to make testing solutions starting at a concentration of 0.49 μM and increasing it by doubling the concentration up to 1000 μM.

Capsiate was extracted from CH-19 sweet (kind gift from Ajinomoto KK, Kawasaki, Japan). Compared with capsaicin, capsiate has an ester bond instead of the amide bond between the vanillyl moiety and fatty acid chain (Figure 1). Harvested chili peppers (CH-19 sweet) were washed and dried. Then the crude oil was extracted from the dried chili peppers using n-hexane. The crude oil was refined by the distillation and the column chromatography. Finally, in order to adjust the concentration, the refined oil was diluted with medium-chain triglyceride. In this original capsiate extract solution, the capsiate content of the sample was ~7%. The rest of the extract solution was mainly caprylic acid. Capsaicin was less than 0.0001% among capsinoids. 70 μl of capsiate extract was dissolved in Tween 80 (1 ml) and ethanol (1 ml), and then dissolved in physiological saline (19 ml) to make a solution of 0.01 M. This solution was diluted with physiological saline to make testing solutions starting at a concentration of 0.49 μM and increasing it by doubling the concentration up to 1000 μM. Capsiate was diluted from the original extract solution every time just before the sensitivity test.

Each subject inhaled a control solution of physiological saline followed by a progressively increasing concentration of capsaicin or capsiate solution. Solutions were inhaled for 15 s every 60 s, by tidal mouth-breathing, while wearing a nose-clip from a Bennett twin nebulizer (3012-60cc; Puritam-Bennett Co., Carsbad, CA, USA). Increasing concentrations were inhaled until five or more coughs were elicited. The nebulizer output was 0.21 ml/min. The cough reflex sensitivities to capsaicin and capsiate were defined as the lowest concentration of capsaicin or capsiate that elicited five or more coughs (C5). In our preliminary experiments, it was confirmed that the Tween 80 and/or caprylic acid dilutions at any concentration used in saline without capsinoids did not induce cough for 15 s inhalation.

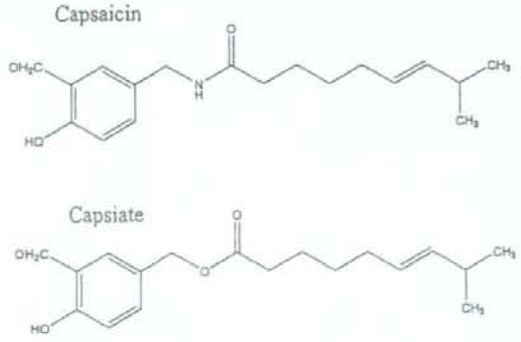


**Figure 1**
Structures of capsaicin and capsiate.

***Oral chemesthesis measurements***

Chemesthesis to capsaicin and capsiate was measured with a modification of the semi-quantitative clinical gustometry using a filter-paper disc, which is routinely used for the evaluation of dysgeusia in a clinical setting [19]. Again, chemesthesis to capsaicin and capsiate were measured on different days. The testing solutions were prepared for both capsaicin and capsiate in the same way as the cough reflex sensitivity measurements, but distilled water was used instead of physiological saline. A droplet of each testing solution was added to the filter paper disc (8 mm diameter), and then the disc was placed on the left side of the tongue 2 cm from the tip (i.e. locus for left cholda tympani nerve), for one second. The filter discs with the progressively increasing concentrations of capsaicin or capsiate were applied every 5 min, and the subject was asked to gargle with distilled water during the interval. Because irritant sensations take longer than classical tastes, subjects were instructed to wait 10 s before making a conclusion on their chemesthesis [16]. The chemesthesis to capsaicin and capsiate were defined as the lowest concentration of capsaicin or capsiate that elicited a pungent or burning sensation for the subject. Although capsinoids have the possibility to elicit bitterness, the subject was asked to ignore the bitterness [20].

In our preliminary experiments, it was confirmed that the Tween 80 and/or caprylic acid dilutions at any comparable concentrations in distilled water without capsinoids did not induce oral chenesthesis, and it was certified that there was no tachyphylaxis of responses to capsinoids with 24-hour intervals for both cough reflex sensitivities oral chemesthesis.

***Statistical analysis***

Results are expressexd as mean ± SE. Comparisons between each threshold concentration in differential stimuli were performed by a paired t-test. Comparisons between the sensitivities in males and females were performed by the Mann-Whitney test. The correlations between each threshold concentration in differential stim-

uli were estimated by Pearson's correlation coefficient. A value of $p < 0.05$ was considered statistically significant.

## Results

Both cough reflex sensitivities and oral chemesthesis tests were performed without any unpleasant feelings or side effects after the tests for all subjects. The mean threshold concentration to induce cough (log $C_5$ value) was significantly greater in capsiate (2.55 ± 0.09 log μM) than in capsaicin (1.20 ± 0.09 log μM) ($p < 0.0001$). The mean threshold concentration to induce oral chemesthesis by capsiate (2.22 ± 0.10 log μM) was significantly greater than that by capsaicin (1.55 ± 0.11 log μM) ($p < 0.0001$). The mean threshold concentration for capsaicin application was significantly greater in cough reflex sensitivity than that in oral chemesthesis ($p < 0.03$).

The mean threshold concentration for capsiate application was significantly greater in cough reflex sensitivity than in oral chemesthesis ($p < 0.01$).

As shown in Figure 2A, there was a strong correlation between capsaicin- and capsiate-induced cough reflex sensitivities ($r = 0.79$, $p < 0.001$). Similarly, as shown in Figure 2B, there was a strong correlation between capsaicin- and capsiate-induced oral chemesthesis sensitivities ($r = 0.64$, $p < 0.01$). These results suggest that cough reflex and pungent sensation are induced by stimulation of TRPV1 in each responsible organ.

However, there was no significant correlation between cough reflex and pungent taste sensitivities induced by capsaicin ($r = -0.12$, $p = 0.50$). Similarly, there was no significant correlation between cough reflex and pungent taste sensitivities induced by capsiate ($r = 0.30$, $p = 0.22$). These results suggest that the same TRPV1 stimulation induce differential strength of sensation according to the organs within individuals.

Table 1 shows cough reflex sensitivities and oral chemesthesis classified by gender. The threshold concentrations to induce cough reflex are significantly greater in males than those in females for both capsaicin and capsiate ($p < 0.03$ and $p < 0.05$, respectively). However, in oral chemesthesis, there were no significant differences between males and females for both capsaicin and capsiate.

## Discussion

In this study, no significant relationship between cough reflex sensitivity and oral chemesthesis to capsinoids within individuals was found. The cough reflex to TRPV1 stimulations are less sensitive in males than in females whereas there was no significant gender difference in the oral chemesthesis to capsinoids. Here we showed that the usefulness of capsinoids with respect to both their action as a tussigen and the capability to evoke oral chemesthesis.

A strong correlation between the threshold concentrations between capsaicin- and capsiate-induced cough was found. Similarly, the threshold concentrations between capsaicin- and capsiate-induced oral chemesthesis significantly correlated. In both sensations, capsiate required a much higher concentration than capsaicin. The intragastric administration of capsiate increases adrenalin secretion and oxygen consumption in mice [21,22]. In addition, capsiate suppresses T cell activation by inhibiting NF-κB-dependent transcriptional activity [23]. These studies suggest that capsiate shares biological activities with capsaicin in spite of very weak pungency. However, the reasons for the weak pungency of capsiate are not clear. Iida and colleagues speculated that less accessibility of capsiate to nociceptors due to its lipophilicity might contribute to the weak pungency [12]. In our studies, the

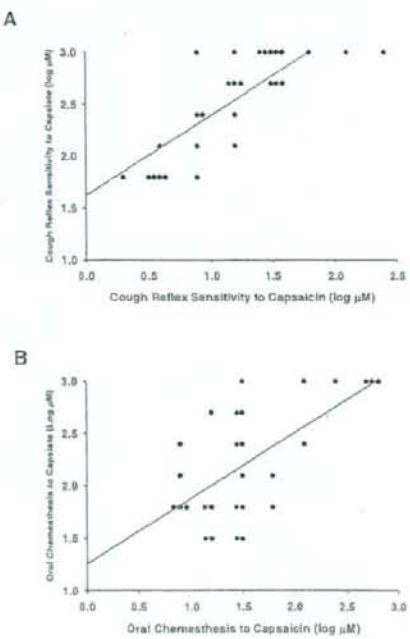


**Figure 2**
Correlations between capsaicin- and capsiate-induced cough reflex sensitivities (A), and between capsaicin- and capsiate-induced oral chemesthesis sensitivities (B). The solid lines represent regression lines.

**Table 1: Gender differences in cough reflex sensitivities and oral chemesthesis**

| | Male | Female | p value |
|---|---|---|---|
| Number | 14 | 14 | |
| Age (year) | 34.2 ± 2.0 | 38.5 ± 4.1 | n.s. |
| Cough reflex sensitivity | | | |
| Capsaicin (Log μM) | 1.41 ± 0.12 | 1.00 ± 0.11 | <0.03 |
| Capsiate (Log μM) | 2.72 ± 0.10 | 2.37 ± 0.13 | <0.05 |
| Oral chemesthesis | | | |
| Capsaicin (Log μM) | 1.51 ± 0.17 | 1.58 ± 0.13 | n.s. |
| Capsiate (Log μM) | 2.22 ± 0.15 | 2.22 ± 0.14 | n.s. |

Data are mean ± S.E. P-values are comparisons between males and females in each variable by the Mann-Whitney test. n.s. denotes not significant.

difference in threshold concentration between capsiate and capsaicin are greater in cough reflex sensitivity than oral chemesthesis. This may reflect lower accessibility to TRPV1 responsible for cough reflex than that for oral chemesthesis.

Individual variations in cough reflex sensitivities were shown in the cough challenge test even in healthy subjects. The variation exists regardless of methods of cough challenge and tussive stimulants. Cough reflex is reportedly less sensitive in men than women [8,9]. Although oral chemesthesis also exhibits variability, a gender difference has not been investigated as far as we know. In our study, the gender difference in cough reflex sensitivities is consistent with previous observations, suggesting methodological appropriateness even with capsiate. We observed no gender difference in oral chemesthesis in healthy subjects using two TRPV1 agonists with different potencies. There are several reports showing an association between oral chemesthesis and taste perception [24,25]. However, the results of the gender difference in taste perceptions are conflicting according to the stimuli and methods [26]. Nasal chemesthesis is relatively better investigated than oral chemesthesis because nasal irritation is an important issue in environmental public health, and data about gender differences are conflicting [27]. In contrast to chemesthesis, gender dependency in pain perception is well documented [28]. Numerous studies demonstrated that certain pain disorders occur with higher prevalence, intensity, or duration in women than in men [29].

The explanation for an increase in cough reflex sensitivity in healthy females is unknown. One hypothesis is an endocrine influence on the cough reflex. Recently, prolactin was reported to enhance TRPV1 response in the presence of estrogen in rat sensory neurons [30]. However, previous studies showing that postmenopausal women have greater cough reflex sensitivity than premenopausal women [8], and more frequently suffer from angiotensin-converting enzyme inhibitor-induced cough [31] would argue against this hypothesis. In addition, our result showing no gender difference in oral chemesthesis may also conflict with the systemic influence of sex hormones on gender differences.

Both the peripheral and central explanations for why oral chemesthesis are not correlated to cough reflex sensitivity are postulated. The lack of relationship between oral chemesthesis and cough reflex sensitivity within individuals may suggest a differential expression of TRPV1 according to the organs within individuals. In patients with chronic cough, increased expression of TRPV1 in airway nerves was reported [15]. Inflammatory bowel disease is associated with the upregulation of TRPV1 in the nerve fibers of the colon [32]. Taste performance on the human tongue varies with the density of fungiform taste buds, which are heavily innervated by chemesthesis receptor neurons [33]. Thus, the organ specific up-regulation of TRPV1 is found in diseases. Differential oral chemesthesis could result from the differential number of TRPV1 in the tongue.

More importantly, the differential sensitivities to capsinoids between cough reflex and oral chemesthesis could be reflected in the differential contribution of indirect activation of afferent neurons. In cough response, capsaicin is known to activate not only C-fibers that have TRPV1 but also rapidly adapting airway mechanoreceptors (PAR) that do not have TRPV1 [17,18]. PAR is activated by a large number of mechanical and chemical irritant stimuli, by inflammatory and immunological mediators, and by airway and lung pathological changes [34]. Presumably, capsaicin activates PAR indirectly by contraction of airway smooth muscle or by an increase in extracellular liquid, or by both mechanisms [34]. Thus, the secondary effect of capsaicin is not small on cough reflex sensitivities. On the other hand, indirect effects of capsaicin on oral chemesthesis sensations have not yet been identified, suggesting that the indirect effect might be negligible in oral chemesthesis.

Besides the peripheral factors, central factors may be involved in the differential sensitivities of TRPV1 stimulation between cough reflex and oral chemesthesis within individuals. In contrast to oral chemesthesis, which was finally integrated by cortical processing, cough reflex is essentially a brainstem reflex. Therefore, there is a possibility that the gain of a cortical neural process is involved in the differences in oral chemesthesis, but not in cough reflex. Evidence of gustatory brainstem taste nuclei and cortical connections, which potentially modulate these processes, provide a plausible neural basis for a central gain mechanism [35,36]. Recently, the possible modifica-

tion of cough reflex by the brain cortex was highlighted [37,38]. There are several studies as to the functions of supramedullary areas responsible for cough. The interaction between sweet taste stimulation and cough reflex was suggested [39]. If the urge-to-cough which precedes coughing was measured, we could more easily understand the lack of relationship between oral chemesthesis and cough reflex sensitivity [40]. Further studies are required to elucidate the relationships between cough reflex and sensory inputs to the cortex.

The lack of relationship between oral chemesthesis and cough reflex sensitivity within individuals might suggest the low possibility of a modulatory effect of capsinoids which were deposited in the oral cavity during the cough challenge test. Although the concentration to induce oral chemesthesis to capsinoids is relatively smaller than that of cough reflex, oral chemesthesis did not trigger cough responses in the present healthy subjects. The lack of gender difference in oral chemesthesis also supports the no modulation hypothesis.

In the present study, we found that the capsiate does not induce the sustained irritant airway feeling that is frequently observed in the case of the capsaicin cough challenge test. This might be attributed to the lipohilicity and instability of capsiate. Although this biophysical feature of capsiate is a disadvantage for the preparation procedure, this could be a benefit for the subject to avoid uncomfortable feelings after the cough challenge test [12].

## Conclusion

In conclusion, the results showed that the sensitivities of sensory afferents were different between cough reflex and oral chemesthesis, suggesting that TRPV1 sensitivities differ among organs within healthy individuals. The results also suggest that capsiate could be a useful tussigen for the cough challenge test.

## Competing interests

The author(s) declare that they have no competing interests.

## Authors' contributions

MY, SE and TE participated the design of the study, collected and analyzed data, and drafted the manuscript. SF, SY, AM and MY participated in the design of the study and collected the data. HA participated in design of the study and helped to draft the manuscript. All the authors read and approved the final manuscript.

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# 人生の最期 やっぱり自宅？

## 男性6割 女性は病院と拮抗

### 配偶者の介護望む

### 家族への迷惑は嫌

人生の最期にみとってほしい場所として男性は六割が自宅を望むのに対し、女性は自宅と病院が四割前後で拮抗（きっこう）する―。終末期に対する意識に男女差があることが、七十歳以上の高齢者を対象にした東北大病院老年科の調査で分かった。男性は「配偶者に介護してほしい」との願望が強く、女性は「家族や友人に迷惑をかけたくない」との回答が多かった。死生観の違いが反映された結果といえそうだ。

調査は二〇〇六年十一月―〇七年四月、岩沼市と川崎市に住む高齢者計二百八十六人から聞き取りして行った。内訳は男性百五十人（平均年齢七十六歳）、女性百三十六人（同七十八歳）。

十五問ある調査項目のうち、「人生の最期をどこでみとられたいか」との質問では、自宅を挙げた人が最多の50㌫。次いで病院34㌫、施設5㌫―の順に多かった。性別にみると、男性は自宅57㌫、病院29㌫、施設5㌫で、女性は自宅43㌫、病院39㌫、施設10㌫だった。

「死に臨んだときに大切なことは」との問いでは（二つ回答）、男女とも同時に充実を図るべきだ」と指摘。調査の中心を担った大学院生山崎都さんは「高齢者だからとひとくくりにせず、個々のニーズに沿ってきめ細かく対応する姿勢が重要だ」と語る。

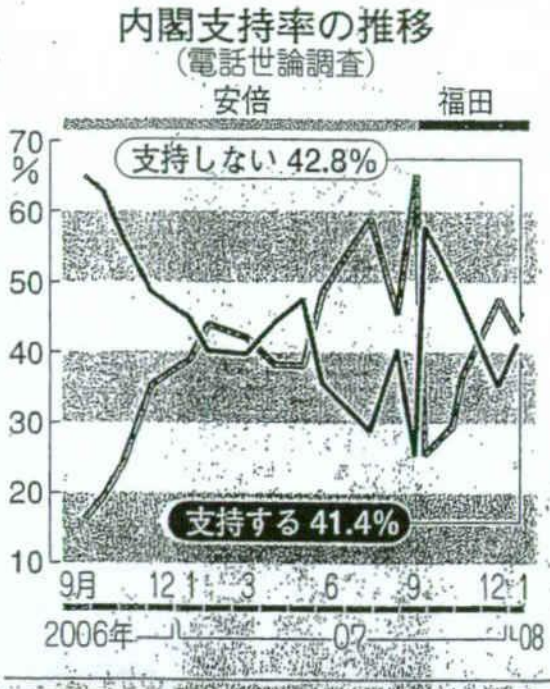
調査結果は、論文にまとめ、米国の老年医学会誌に近く掲載される。

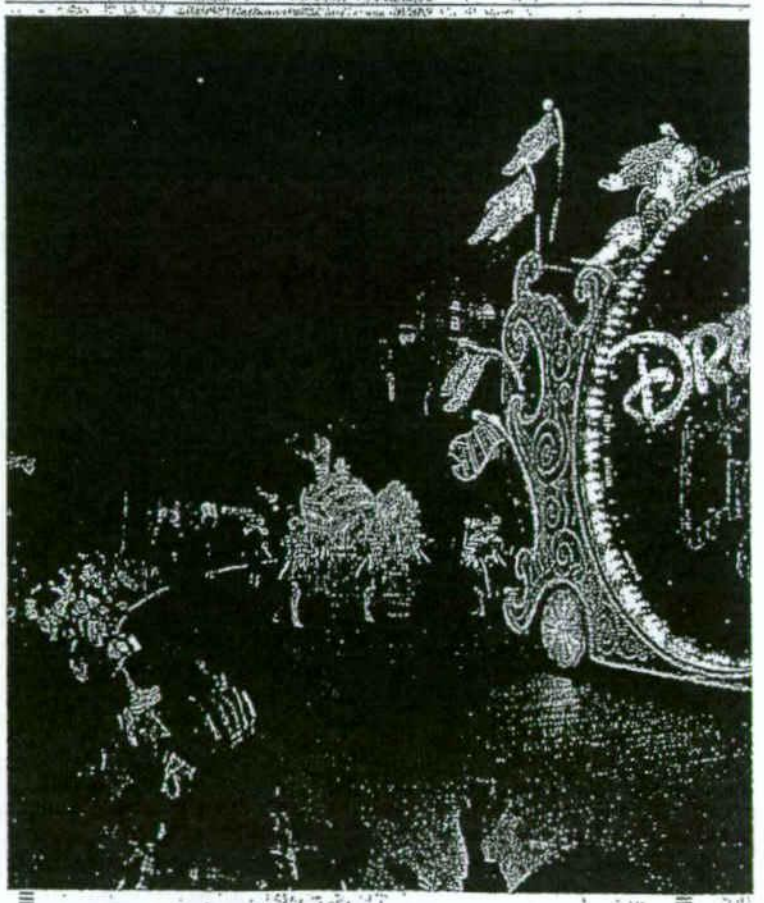
**？ 終末期医療に関する調査** 厚生労働省の検討会が2004年に公表した調査では、20歳以上の国民の58・8㌫が「できるだけ自宅で療養したい」と答えた。人口動態調査によると、実際の死亡場所は、1960年代までは7割以上が在宅死だったが、現在は病院死が8割を超えている。

### ―70歳以上対象に東北大調査―

終末期の過ごし方をめぐる調査は、若年層を含めた幅広い世代を対象としたものがほとんど。今回のように、高齢者に限定した調査は珍しい。

調査は二〇〇六年十一月―〇七年四月、岩沼市と川崎市に住む高齢者計



再開された東京ディズニーランドのパレード＝12日夜、千葉県浦安市

# 専門職大学院 4割の専攻 充足率 分㌫

高度な専門的知識を身に付けた職業人の養成を目的に、二〇〇六年四月までに開設した国公私立と株式会社立の「専門職大学院」＝❓＝四十九校計六十六専攻（法科大学院を除く）のうち、約四割に当たる二十五専攻が定員割れになっていることが十一の調査で分かった。文科省は「いれば設が、設置の質が社られないなけ難しい」。

定員割は、分野ネス・技が二十八攻（32㌫専攻のう㌫）、公のうち一一方、公産、原子

この日午後七時半、「東 使用した。 地面に落ちた

# 「最期は病院で」女性に多く

## 東北大病院 高齢者調査

## 「周りに迷惑かけない」重視

「最期は自宅で看取られたい」と願う70歳以上の女性は同年代の男性より少なく、逆に病院で最期を迎えたいと考えている女性が男性より多いことが、東北大病院の調査でわかった。人生を終えるに当たって周囲に迷惑をかけないように気遣う人も、男性より女性に目立つ。

同病院老年科は06年11月～07年4月、川崎市と宮城県岩沼市の老人クラブに所属する人を中心とする70歳以上の男女計286人に、聞き取りによるアンケートで15項目を質問した。男性は150人で平均76歳、女性は136人で同78歳。都市部と農村部で回答の傾向に違いはなかった。

「人生の最期をどこで看取られたいか」という質問に対し、最も多かった答えは「自宅」の50%。次いで「病院」34%、「施設」5%だった。ただし性別によって違いが顕著で、「自宅」は男性が57%に対し、女性43%。「病院」も男性が29%に対して女性40%だった。

「死に臨んだ時に大切なこと」を二つ挙げてもらうと、男女ともに1位は「安楽に死ぬこと」で110人前後が選んだが、次点は男性が「家で死ぬこと」(77人)、女性は「家族や友人に迷惑をかけないこと」(58人)だった。

特に女性の場合、「家族や友人に迷惑をかけない」を選んだ人は、「安楽に死ぬこと」と答えた人より、病院での死を望む傾向が強かった。

「誰に介護してほしいか」は、男性の7割が「配偶者」、女性は「娘」(25%)、「嫁」(17%)、「配偶者」「病院」(ともに16%)などと分かれた。(高橋美佐子)

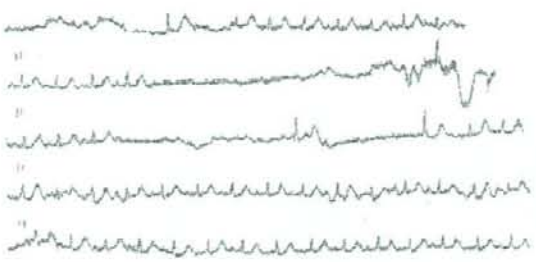


Figure 1. Rhythm strip (25 mm/s and 1 cm/mV): Mobitz type II atrioventricular block with cardiac pauses.

up to 5 seconds due to a Mobitz type II block without reliable escape rhythm (Figure 1).

She was transferred to the cardiac care unit, where her rhythm disorder could be better monitored. Continuous cardiac monitoring revealed that all screaming episodes occurred during cardiac pauses. The echocardiogram showed a calcified mitral valve with stenosis and aortic valve sclerosis and insufficiency.

The cardiac pauses also occurred during sleep, one lasting up to 16 seconds. Consequently, a ventricle-paced ventricle-sensed inhibited (VVI) pacemaker was implanted, after which the patient's screaming stopped.

In conclusion, a cardiac rhythm disorder caused screaming in this patient and was probably directly connected to presyncopal feelings, which were provoked by second- and third-degree atrioventricular blockades with unreliable escape rhythm superimposed on a sick sinus syndrome. Initially, no transient rhythm changes could be assessed.

This woman's history emphasizes that screaming in older people should be taken seriously, because it may indicate severe disease. It also underscores that severe cardiac diseases may present with atypical symptoms such as screaming in geriatric patients.[5] A literature search was conducted, but no single case history of screaming based on cardiac pathology was found. Nevertheless, cardiac arrhythmias should be part of a differential diagnosis for vocalization behavior, especially when presyncopal feelings accompany this behavior. ECG should be performed routinely, and continuous electrocardiographic monitoring should be considered if the cause of screaming remains unclear. Psychiatric or cognitive comorbidity should not exclude cardiological examination, because screaming in older people may really be a "cri de coeur."

*Justi Ernst, MD*
*Department of Geriatrics*
*Radboud University Medical Centre*
*Nijmegen, The Netherlands*

*Chris J. M. Hilkens, MD*
*Department of Geriatrics*
*Canisius Wilhelmina Hospital*
*Nijmegen, The Netherlands*

*Joep L. R. M. Smeets, MD, PhD*
*Department of Cardiology*
*Willibrord H. L. Hoefnagels, MD, PhD*
*Marcel G. M.Olde Rikkert, MD, PhD*
*Department of Geriatrics*
*Radboud University Medical Centre*
*Nijmegen, The Netherlands*

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## CONTRIBUTION OF GASTRIC ACID IN ELDERLY NURSING HOME PATIENTS WITH COUGH REFLEX HYPERSENSITIVITY

*To the Editor*: Cough is not only an important defense mechanism for the airway, but also presents as one of the most common symptoms for patients who seek medical care. A weak cough reflex is one of the responsible factors for aspiration pneumonia in older people,[1,2] whereas persistent cough, whether during the day or night, is also a problem in older people. The major causes of persistent cough in adults are postnasal drip, asthma, and gastroesophageal reflux disease (GERD).[3] In older people, gastroesophageal reflux (GER) as a cause of persistent cough is difficult to detect. Although a symptom of cough may reflect the present condition even in older people, the prevalence of cough reflex hyper- or hyposensitivity in institutional older people has not been reported. Additionally, management of persistent cough in older people with physical and mental status that has deteriorated has received less focus. Therefore, a cross-sectional study was conducted from August to September 2004 for the distribution of cough reflex sensitivity in institutional older peple. The criterion for patient selection was that physical symptoms and cognitive impairment must have been stable for the preceding 3 months. Participants with chronic sinusitis or

respiratory disease or those who were immunocompromised, were current smoker, or were taking drugs influencing airway reactivity or lower esophageal sphincter tone or antisecretory gastric treatments were excluded. Consequently, 123 eligible patients of 132 institutionalized patients were recruited. Informed consent was obtained from them or their families before the study started. All eligible participants (38 men, mean age ± standard deviation 84.4 ± 5.6, mean Mini-Mental State Examination score 18.7 ± 7.2,[4] and mean Barthel Index 74.1 ± 10.6[5]) were examined for individual cough threshold ($PC_5$),[6–8] determined as the concentration of citric acid solution at which the patient coughed at least five times after a 1-minute inhalation using an ultrasonic nebulizer. Thereafter, participants judged as cough-reflex hypersensitive underwent chest x-ray, esophageal endoscopy, or a 24-hour pH monitoring[9] to investigate the cause of cough. It was impossible to examine four of the participants judged to be cough-reflex hypersensitive using esophageal endoscopy or a pH monitoring system because of their poor physical and mental status. No participant could precisely answer a questionnaire related to symptoms of GERD because of poor cognitive and physical functions. Second, a 1-month randomized, controlled trial was conducted for 28 days in elderly residents with cough-reflex hypersensitivity to investigate an empirical effect of proton pump inhibitors (PPIs) on cough-reflex hypersensitivity. They were randomly assigned to the PPI-treated group (lansoprazole 15 mg/d) or the control group (placebo 1 tablet/d), using a random-numbers table and were investigated for change in cough-reflex sensitivity. The local ethical committee in the nursing home approved the protocol.

The distribution of the number of patients at each concentration of cough-reflex sensitivity was trimodal, with peaks located at 2.8, 11.3, and 180 mg/mL as $PC_5$ (Figure 1). Accordingly, cough reflex sensitivity was divided into three groups: hypersensitive ($PC_5 \leq 2.8$ mg/mL), intermediate sensitive (2.8–45.0 mg/mL), and hyposensitive ($PC_5 \geq 45.0$ mg/mL). The numbers of patients with hypersensitive, intermediate, and hyposensitive cough reflexes were 20 (16.3%), 70 (59.3%), and 30 (24.4%), respectively. There was no significant difference in age between the groups.

Sixteen of 20 participants with cough-reflex hypersensitivity who were examined had some findings of esophageal hiatus hernia and GER or esophagitis. Three of 16 participants were defined as grade A according to the Los Angeles classification for GERD.[10] Six of 13 participants not defined as having GERD underwent 24-hour pH monitoring of the upper gastroesophageal tract, and GER was diagnosed if esophageal pH was less than 4.0 for longer than 4.9% of total study time.[11] All six fit the criteria for GER, and their esophageal pH was less than 4 for a mean of 7.05% of the time that they were being monitored. All of participants had esophageal hiatus hernia.

In the PPI trial study, there was no significant difference between groups in sex, age, or $PC_5$ at Day 0. The intervention of lansoprazole medication for 4 weeks raised $PC_5$ significantly from a low to a healthy range (1.7 ± 0.7 to 14.5 ± 6.0 mg/mL) in patients with cough-reflex hypersensitivity ($P<.001$). In contrast, in the placebo group, there was no significant difference in $PC_5$ between Day 0 and Day 28. In a comparison between the groups at Day 28, $PC_5$ in the PPI-treated group was significantly higher than that of the control group ($P<.001$).

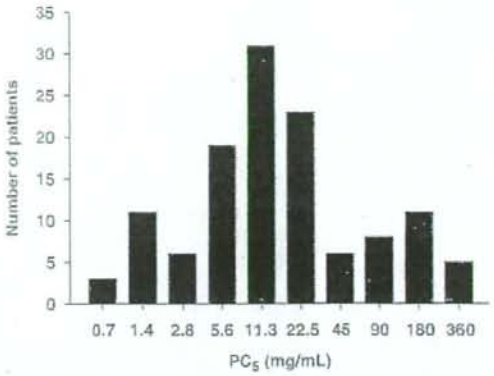


Figure 1. The distribution of cough-reflex sensitivities in older people in nursing homes. $PC_5$ = individual cough reflex sensitivity, determined as the concentration of citric acid solution at which the patient coughed at least five times.

In conclusion, the prevalence of older people with cough reflex sensitivity in nursing homes is similar to that of older people with cough-reflex hyposensitivity. Medication with PPI for 1 month is effective against cough-reflex hypersensitivity in older people, probably due to GER.

*Satoru Ebihara, MD, PhD*
*Takae Ebihara, MD, PhD*
*Miyako Yamasaki, MD*
*Masanori Asada, MD, PhD*
*Shinsuke Yamanda, MD*
*Kaijun Niu, MD, PhD*
*Hidetada Sasaki, MD, PhD*
*Hiroyuki Arai, MD, PhD*
*Department of Geriatrics and Respiratory Medicine*
*Tohoku University School of Medicine*
*Sendai, Japan*

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**Author Contributions:** Satoru Ebihara: study design, interpretation of data, preparation manuscript. Takae Ebihara: study concept, acquisition of subjects, interpretation data: Miyako Yamazaki, Masanori Asada, Shinsuke Yamanda, Niu K: acquisition of subjects. Hidetada Sasaki: study concept, supervision. Hiroyuki Arai: interpretation of data, preparation manuscript, and supervision.

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## HIP FRACTURE AND DEPRESSION IN ELDERLY PATIENTS: IS THERE A SEX EFFECT?

*To the Editor:* We read with great interest the articles by Burns et al.[1] and Lenze et al.[2] and the related editorial by Kamholz and Unützer[3] on hip fracture and depression.

The problem is important, because depression is a highly disabling condition in itself, and when it is associated with hip fracture, it has a profound effect on prognosis.

Thankfully, depression is a relatively modifiable condition no matter whether it is a cause or result of disability, but until now, there have been no studies identifying predictors of depression post-hip fracture and none that address methods of prevention and intervention.[3]

We discuss data taken from a population of 766 elderly patients not affected by severe dementia (26.2% men; mean age ± standard deviation 78.8 ± 7.6) consecutively admitted to the Geriatric Evaluation and Rehabilitation Unit (GERU), P. Richiedei Hospital, Gussago, Brescia, Italy. In particular, we present data emphasizing the role of sex as a risk factor for depression after an important negative event such as hip fracture.

Only first admissions that took place over the study period were considered.

Admission to the GERU was based on the clinical judgment of potential benefit of a multidimensional assessment and rehabilitation programs. Patients in immediate and obvious need of nursing home placement were excluded. Only subacute patients were selected for admission, and those with acute conditions were excluded. The most prevalent diagnoses on discharge were musculoskeletal disorder (72%), ischemic heart disease (56%), chronic obstructive pulmonary disease (44%), hip fracture (9%), and parkinsonism (7%). The mean length of stay was 29.6 ± 13.0 days.

Two trained staff geriatricians performed a multidimensional evaluation, including information on demographics (age, sex, education, living site before admission and after discharge, living conditions, and caregiver or formal support availability), cognitive and affective status, physical health, functional abilities, and social support on the third day after admission using a standard protocol. Comorbidity was assessed using the Charlson Index,[4] cognitive status using the Mini-Mental State Examination (MMSE),[5] depressive symptoms using the Geriatric Depression Scale (GDS, 30 items),[6] malnutrition using the Prognostic Nutritional Index (PNI, score <40),[7] and disability using the activity of daily living scale.[8] Depression was considered present in patients with a MMSE score greater than 12 out of 30, clinically depressed at the psychiatric interview, and scoring 15 or higher on the GDS.

Table 1. Association Between Hip Fracture and Depression in 766 Elderly Patients Admitted to a Geriatric Rehabilitation Unit

| | Total (N = 766) | Women (n = 565) | Men (n = 201) |
|---|---|---|---|
| Fracture Status | n/N (%) | | |
| Not fractured | 286/623 (45.9) | 219/439 (49.9) | 67/184 (36.4) |
| Fractured | 68/143 (47.5) | 57/126 (45.2) | 11/17 (64.7) |

Data are expressed as number of depressed patients over the total number of subjects for each specific group.

The association between hip fracture and depression is reported in Table 1 and shows a peculiar sex effect. In the female group admitted with hip fracture, the prevalence of depression was not different from that found in those admitted for other reasons (45.2% vs 49.9%). However, male patients with hip fracture had almost two times the prevalence of depression as those admitted for other conditions (64.7% vs 36.4%).

Depressed men and women with hip fracture were also had different MMSE scores (18.9 ± 7.1 vs 23.6 ± 2.6) and Charlson Index (1.2 ± 1.6 vs 0.9 ± 0.9), whereas no differences were detected between groups in terms of age, education, living alone, serum albumin, or PNI.

Data show differences between men and women in terms of suffering depression after hip fracture, a trend previously suggested.[9] Men had also more cognitive impairment and more comorbidity than women, indicating a more-compromised health status.

Men and women who incur a hip fracture have higher risks of dying or being institutionalized within 2 years than their peers; the independent effect of hip fracture on this outcome is significantly greater for men than women.[10] The data from the current study may at least partially explain this sex specificity, because depression may be the most important factor linking hip fracture with mortality. From this perspective, sex specificity could be an important issue in the studies on predictors of depression after hip fracture.


*Renzo Rozzini, MD*
*Alessandro Morandi, MD*
*Intissar Sleiman, MD*
*Department of Internal Medicine and Geriatrics*
*Poliambulanza Hospital*
*Brescia, Italy*
*Geriatric Research Group*
*Brescia, Italy*

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*Cardiovascular, Pulmonary and Renal Pathology*

# Macrophage Colony-Stimulating Factor Improves Cardiac Function after Ischemic Injury by Inducing Vascular Endothelial Growth Factor Production and Survival of Cardiomyocytes

Tatsuma Okazaki,* Satoru Ebihara,* Masanori Asada,* Shinsuke Yamanda,* Yoshifumi Saijo,† Yasuyuki Shiraishi,† Takae Ebihara,* Kaijun Niu,* He Mei,* Hiroyuki Arai,* and Tomoyuki Yambe†

*From the Department of Geriatrics and Gerontology,* Tohoku University School of Medicine, Sendai; and the Department of Medical Engineering and Cardiology,† Institute of Development, Aging, and Cancer, Tohoku University, Sendai, Japan*

**Macrophage colony-stimulating factor (M-CSF), known as a hematopoietic growth factor, induces vascular endothelial growth factor (VEGF) production from skeletal muscles. However, the effects of M-CSF on cardiomyocytes have not been reported. Here, we show M-CSF increases VEGF production from cardiomyocytes, protects cardiomyocytes and myotubes from cell death, and improves cardiac function after ischemic injury. In mice, M-CSF increased VEGF production in hearts and in freshly isolated cardiomyocytes, which showed M-CSF receptor expression. In rat cell line H9c2 cardiomyocytes and myotubes, M-CSF induced VEGF production via the Akt signaling pathway, and M-CSF pretreatment protected these cells from $H_2O_2$-induced cell death. M-CSF activated Akt and extracellular signal-regulated kinase signaling pathways and up-regulated downstream anti-apoptotic Bcl-xL expression in these cells. Using goats as a large animal model of myocardial infarction, we found that M-CSF treatment after the onset of myocardial infarction by permanent coronary artery ligation promoted angiogenesis in ischemic hearts but did not reduce the infarct area. M-CSF pretreatment of the goat myocardial infarction model by coronary artery occlusion-reperfusion improved cardiac function, as assessed by hemodynamic parameters and echocardiography. These results suggest M-CSF might be a novel therapeutic agent for ischemic heart disease. *(Am J Pathol 2007, 171:1093–1103; DOI: 10.2353/ajpath.2007.061191)***

The administration of angiogenic growth factors such as vascular endothelial growth factor (VEGF) is an innovative strategy to treat myocardial ischemia. VEGF has been used in animal models and in clinical trials of myocardial ischemia to develop growth of collateral blood vessels and to promote myocardial perfusion, and its therapeutic potential has been reported.[1–3] Hematopoietic growth factors are potent therapeutic agents for myocardial infarction. Erythropoietin improved cardiac function after myocardial infarction.[4,5] Granulocyte colony-stimulating factor (G-CSF) improved cardiac function and prevented cardiac remodeling after myocardial infarction.[6] A combination of stem cell factor and G-CSF treatment improved cardiac function and survival after myocardial infarction.[7] Macrophage colony-stimulating factor (M-CSF) in combination with G-CSF improved ventricular function after myocardial infarction in rats, but few results were shown by M-CSF treatment alone, and their mechanism was not defined.[8] Moreover, to estimate growth factor-induced therapeutic angiogenesis in hearts, large animal models are necessary,[3] but the effects of M-CSF in large animal models have not been reported. M-CSF has been initially characterized as a hematopoietic growth factor, and has been used to prevent severe infections in myelosuppressed patients after cancer chemotherapy.[9,10] M-CSF stimulates the survival, prolifera-

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Current address of T.O.: Department of Anatomy, University of California–San Francisco, San Francisco, CA.

Address reprint requests to Satoru Ebihara, M.D., Ph.D., Department of Geriatrics and Gerontology, Tohoku University School of Medicine, Seiryo-machi 1-1, Aoba-ku, Sendai 980-8574, Japan. E-mail: s_ebihara@geriat.med.tohoku.ac.jp.