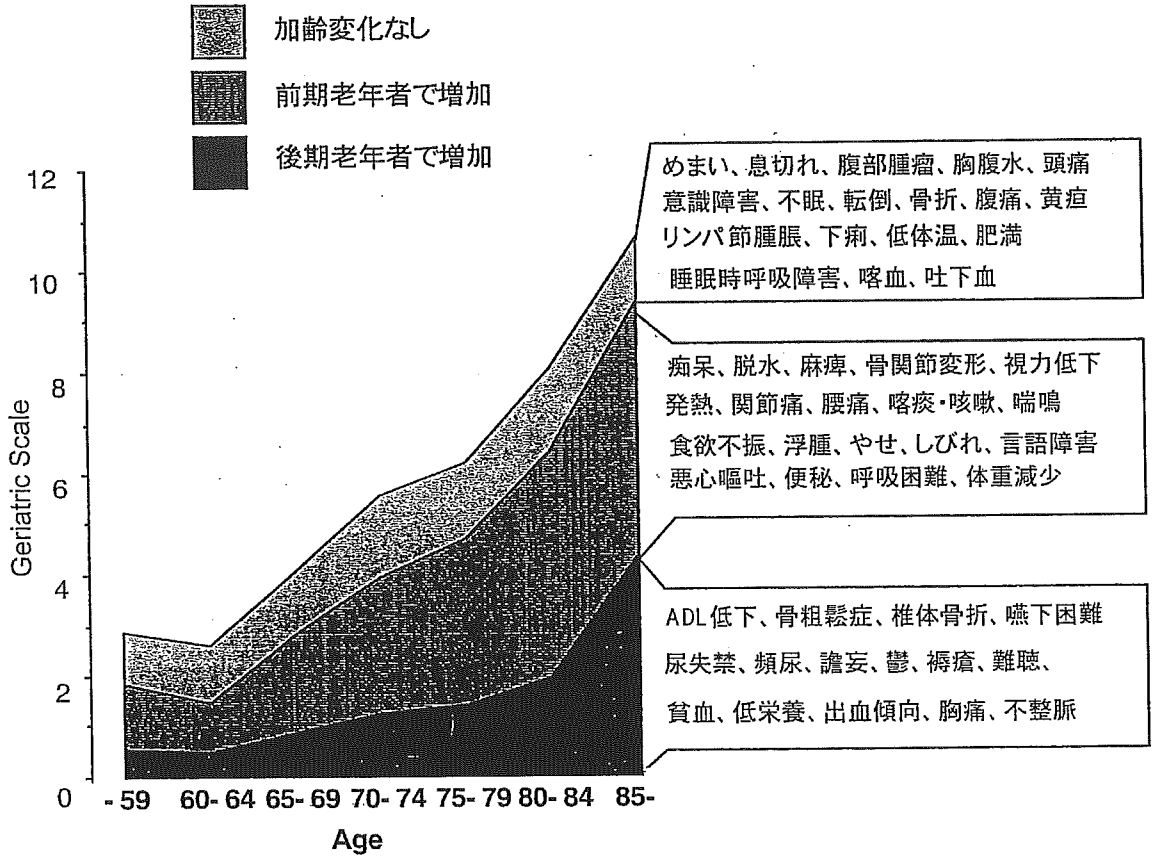

図1　3つの老年症候群

痰，喘鳴）などに大別される．頻度の極端に少ないものは吐き気（5％），肥満（0％）であった．

このように，痴呆は他の代表的な老年症候群である尿失禁，転倒骨折，誤嚥性肺炎，低栄養，廃用性症候群などを高頻度に合併し，さらに譫妄やうつなども問題となる複雑な医療分野といえる．

2．機能評価を行い機能低下の早期発見をする

痴呆の重症度では，自立困難や日常生活動作の困難などが判定で重要視されるが，治療効果では「記憶検査」以外長く省みられることがなかった．進展予防に有効な薬剤が開発され，記憶力の保持には著明な効果が見られないことが分かって，ようやくこれらを加味した評価が取り入れられるようになった．

高齢者の総合的機能評価はもともと，認知能だけでなく，うつ，ADL，生活自立，家庭環境，サービス利用，介護負担などを総合的に評価するものであり，老年医学における痴呆医療の最も重要な領域である．

杏林大学高齢医学では，物忘れ外来開設以来全例に総合的機能評価を施行し，治療判定に役立てている．これまでの成績では，薬物療法（塩酸ドネペジルなど）や行動療法（回想法，オリエンテーション療法，運動療法）などで，最も改善効果が強い機能は，生活自立と関連する手段的ADL[3]であった．手段的ADLは，交通手段を使って外出する，買い物をする，電話をかける，金銭管理をする，服薬管理をする，炊事をする，掃除などの家事をする，洗濯をするの8項目であり，在宅の生活自立に直結している．これに次いで感度のよい機能評価は，短期記憶力（HDSR, MMSE）であり，問題行動28項目の調査（痴呆行動障害尺度）は介護負担と良い相関があるが，

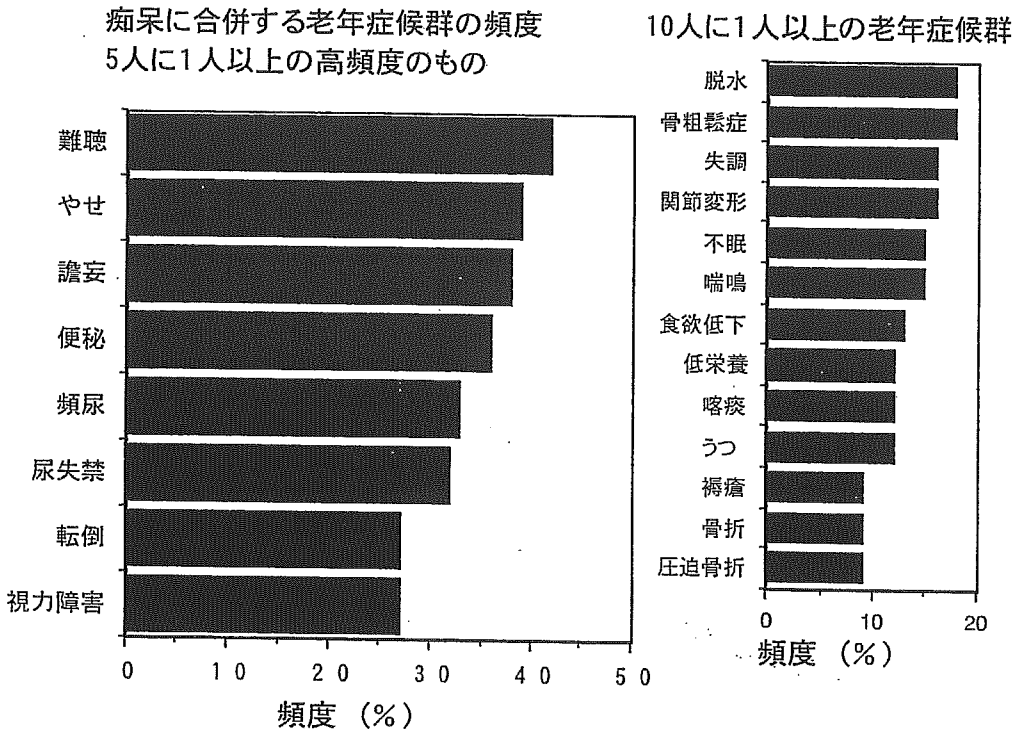

図2 合併する老年症候群の頻度

症例によって改善と不変に分かれ，今後の検討課題である．

3. 寝たきりのケアを評価する

今後痴呆のケア技術は急速に発展し，いいケアをして良くなったことを客観的に評価して介護者に報いることである．そのためには，重症痴呆患者のQOLを評価する手技が必要である．意欲の指標（表1）は痴呆の程度や生命予後と強い相関をもち（図3），このような需要に最も的確に応える指標と考えられる[4]．また，今後重症痴呆の言語，非言語能力を定量的に評価する手技の開発と応用も，高齢者医療の重点領域である．

4. ねたきり予防

寝たきり高齢者が100万人を越え大きな国民的課題であるが，最近の東京都の調査では，脳卒中や骨折などの後，そのまま寝たきりになるのは3分の1に過ぎず，残りは寝たきりの直接間接の原因や寝たきりになっていく過程が不明なままである．寝たきりプロセスの解明と，早期発見のための寝たきりリスクチェック表の開発，医療福祉政策に反映しうる実効性のある，寝たきりを減らす介入方法の実証研究が必要である．本講演では寝たきりプロセスの解明と，これに立脚した医療福祉政策として実現可能な有効性のある寝たきり予防研究の最近の展開を紹介したい．

対象と方法の概要は以下の通りである．

1）施設入所高齢者1964名に対し，継続して転倒，ADLなどの縦断的調査．

2）15施設のグループホームのADL，痴呆，問題行動の縦断的変化の測定．

3）全国9市町地域高齢者（12000名）——愛媛県大三島町，熊本県相良町，高知県香北町，京都府園部町，滋賀県余呉町，北海道浦臼町，福岡県自治体，宮城県仙台市，群馬県中之条町の機能変化．

4）体操会員8000名に対し，活力度調査（36項目）

表1　意欲の指標　Vitality Index

1) 起床（Wake up）	
いつも定時に起床している	2
起こさないと起床しないことがある	1
自分から起床することがない	0
2) 意志疎通（Communication）	
自分から挨拶する，話し掛ける	2
挨拶，呼び掛けに対し返答や笑顔がみられる	1
反応がない	0
3) 食事（Feeding）	
自分で進んで食べようとする	2
うながされると食べようとする	1
食事に関心がない，全く食べようとしない	0
4) 排泄（On and Off Toilet）	
いつも自ら便意尿意を伝える，あるいは，自分で排尿，排便を行う	2
時々尿意，便意を伝える	1
排泄に全く関心がない	0
5) リハビリ，活動（Rehabilitation, Activity）	
自らリハに向かう，活動を求める	2
促されて向かう	1
拒否，無関心	0

除外規定；意識障害，高度の臓器障害，急性疾患（肺炎などの発熱）
判定上の注意：1）薬剤の影響（睡眠薬など）を除外．起座できない場合，開眼し覚醒していれば2点．2）失語の合併がある場合，言語以外の表現でよい．3）器質的消化器疾患を除外．麻痺で食事の介助が必要な場合，介助により摂取意欲があれば2点（口まで運んでやった場合も積極的に食べようとすれば2点）．4）失禁の有無は問わない．尿意不明の場合，失禁後にいつも不快を伝えれば2点．5）リハビリでなくとも散歩やリクリエーション，テレビなどでもよい．
寝たきりの場合，受動的理学運動に対する反応で判定する．

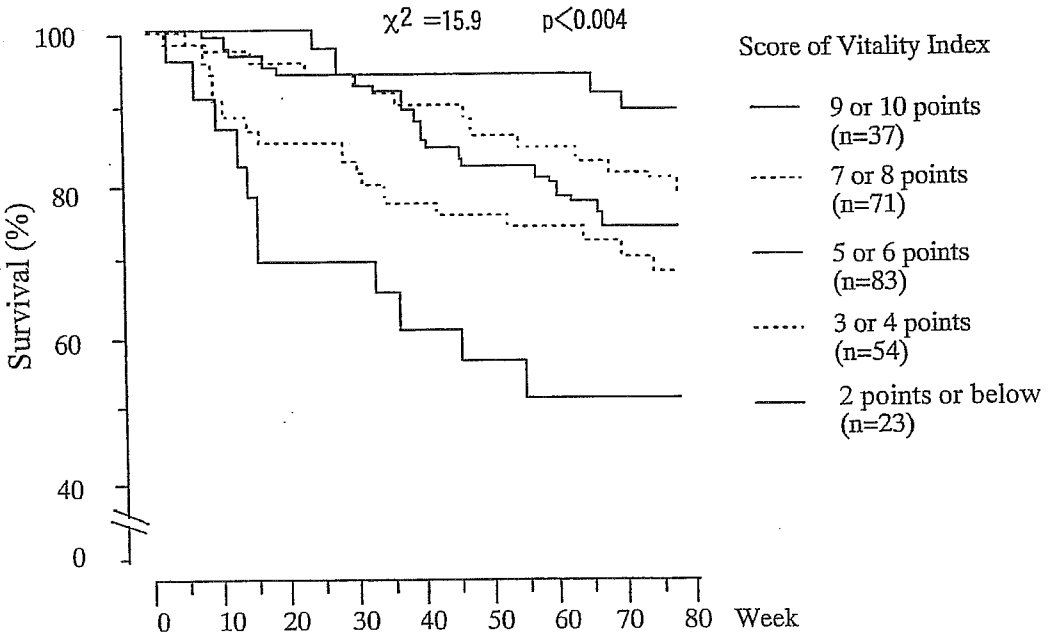

図3　Vitality Index and Survival

寝たきりプロセスの解明と予防因子の抽出：

地域縦断調査2000名から，脳血管障害，痴呆，転倒，うつなどの危険因子と，飲酒，長寿教室への参加などの，ADL低下予防因子が抽出された．脳血管障害に関しては，西永は，家庭血圧148 mmHg以上で要介護率が4倍以上になることが示された．

特養，老健入所者1176名の縦断調査を施行し，開始時のADLに関する意欲（意欲の指標）の低下は寝たきり度（JABCランク）の悪化予測因子

として最も有用であった。

急性期病院における高齢者のADL低下要因として，70歳までの加齢，入院時ADL，緊急入院の有無は関連がなく，90歳以上の高齢，多病，低体重，痴呆，意欲の低下が危険因子として抽出された。

寝たきり過程の促進因子では，介護施設におけるADLや自立度低下に関し，重要な徴候は，転倒，大腿骨頸部骨折，麻痺，息切れが最も重要で，ついで，感染症徴候（発熱），痴呆の進行に注意すべきである。

この中で，大腿骨頸部骨折は意欲が保持されており，早期のリハビリが重要と考えられた。また転倒を繰り返すと意欲が低下することが判明した。

施設縦断調査1194名により，骨折率は年間2.7％（32件）であった。

転倒に関する危険予測因子として，在宅住民調査により，下肢筋力低下，柔軟性減少，バランス不安定，重心動揺の増大，歩行時つま先が上がらないなどが抽出され，下肢筋力強化，歩行によるバランス獲得，靴の工夫などが転倒予防に資することが判明した。

意欲の減退に関しては，転倒，痴呆，食欲不振が有意な要因であった。

危険因子に対する介入：

転倒予防

転倒スコア（仮称）の策定と有用性の検討：相良村，浦臼町，杏林大学，東北大学，高知医大など在宅，外来における大規模調査を実施した。スコアと転倒（既往）とは，正の良好な関係が得られた。重回帰分析及び因子分析では，主として歩行機能，運動機能，コミュニケーション能力が有意な因子で，環境要因の関与は少なかった。

運動の効果

高齢者を取り巻く介護力が向上することで，各ADL項目の自立度が向上するという知見は，早期に適切な介入を行うことが，短期的にも介護予防に効果的であることを示唆している。

閉じこもりを防ぐための，高齢者の閉じこもりと，生活における楽しみの調査において，散歩，動物の世話，老人クラブへの参加が閉じこもりの予防に対して有用な活動である可能性が示された。

全国規模の体操教室調査により（縦断調査の1年目），運動の心身面への好影響，適切な運動量による寝たきり予防効果が示唆された[5]。

文　献

1）鳥羽研二：シンポジウム　介護保険と高齢者医療：施設介護の問題点．日本老年医学会雑誌　34(12)：981-986, 1997

2）鳥羽研二，大内尉義：老年症候群の特徴と検査の重要性．総合臨床，47, 1, 1998

3）Lawton, M.P & Brody, E.M.: Assessment of older people: Self-Maintaing and instrumental activities of daily living. Gerontologist: 9, 179-188, 1969

4）Kenji Toba, Ryuhei Nakai, Masahiro Akishita, et al.: Geriatrics Gerontology International 2: 23-29, 2002

5）厚生労働省効果的医療技術の確立推進研究「寝たきりプロセスの解明と主たる因子に対する介入研究」班（班長：鳥羽研二），平成15年度報告書

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Adiponectin Replacement Therapy Attenuates Myocardial Damage in Leptin-deficient Mice with Viral Myocarditis

T TAKAHASHI[1], S SAEGUSA[1], H SUMINO[3], T NAKAHASHI[2], K IWAI[2], S MORIMOTO[2] AND T KANDA[1]

[1]Department of General Medicine and [2]Department of Geriatric Medicine, Kanazawa Medical University, Ishikawa, Japan; [3]Second Department of Internal Medicine, Gunma University School of Medicine, Gunma, Japan

The effects of adiponectin replacement therapy on myocardial damage were studied in leptin-deficient (OB) mice with acute viral myocarditis. Encephalomyocarditis virus was injected intraperitoneally into OB and wild-type (WT) mice. One subgroup of OB mice received no intervention and another subgroup received daily adiponectin replacement, simultaneously with viral inoculation. Differences in heart weight, cardiac histological score, numbers of infiltrating or apoptotic cells in the myocardium and the immunoreactivity of adiponectin receptors in myocytes were determined. The reactivity of adiponectin receptor 1 in myocytes from OB mice on day 4 and day 8 after viral inoculation was significantly decreased compared with that in myocytes from WT mice; the OB mice also had elevated cardiac weights and severe inflammatory myocardial damage. Adiponectin replacement in OB mice inhibited the development of severe myocarditis by augmenting myocyte adiponectin receptor 1 reactivity. Exogenously administered adiponectin may inhibit the progression of viral myocarditis through binding to the adiponectin receptor 1 in leptin-deficient conditions.

KEY WORDS: ADIPONECTIN; VIRAL MYOCARDITIS; LEPTIN DEFICIENCY; MYOCARDIAL DAMAGE

Introduction

Several studies have reported an association between leptin and cardiovascular conditions such as hypertension and chronic heart failure with cachexia. Reduced leptin concentrations may diminish the degree of cardiac adaptation in heart failure,[1] and plasma leptin levels were inappropriately low in patients with cachectic chronic heart failure.[2] We have recently found that leptin deficiency enhanced myocardial necrosis and lethality in a mouse model of viral myocarditis. However, leptin replacement inhibited the development of severe myocarditis, suggesting a protective role for leptin against myocyte damage,[3] although the mechanisms involved are unclear.

Adiponectin, also known as adipocyte complement-related protein of 30 kDa,[4] is a cytokine secreted by adipocytes that has anti-diabetic and anti-atherogenic effects.[5] Concentrations of adiponectin in blood are diminished in obesity, insulin resistance and type 2 diabetes.[4] The adiponectin gene and the obese gene, which encodes leptin, show several striking similarities in humans.[6] Both genes are composed of three exons and have a long first intron, and are expressed specifically in adipose tissues.[6] Adiponectin and leptin control fuel homeostasis, body weight and insulin sensitivity. Amelioration of insulin resistance, pancreatic β-cell degranulation and diabetes after crossing leptin-deficient mice with globular domain adiponectin transgenic mice has been described, indicating that globular adiponectin and leptin may have overlapping functions.[5] Thus, adiponectin may also possess functions similar to those of leptin in the development of heart failure.

In this study, we hypothesized that adiponectin could play a protective role against the progression of severe viral myocarditis in leptin deficiency. We examined the effects of adiponectin replacement therapy on myocardial damage in leptin-deficient mice with acute viral myocarditis.

Materials and methods

INFECTION PROTOCOL

Six-week-old female leptin-deficient *ob/ob* mice and C57BL wild-type mice were obtained from the Jackson Laboratory (Bar Harbor, ME, USA). A myocarditic variant of the encephalomyocarditis (EMC) virus was obtained from Dr Y Seto (Keio University, Tokyo, Japan). The viral preparations were stored at –80 °C in Eagle's minimum essential medium supplemented with 0.1% fetal bovine serum. Ethical approval for this study was obtained from the animal experimental committee in Kanazawa Medical University. All animals were treated in accordance with the Kanazawa Medical University guidelines for the care and use of laboratory animals. All animals were inoculated intraperitoneally with 500 plaque-forming units of EMC virus suspended in 0.1 ml of saline.

TREATMENT PROTOCOL

The leptin-deficient mice were randomly assigned to one of two groups. The first group did not receive interventional therapy (OB group). The second group received daily subcutaneous injections of recombinant mouse full-length adiponectin (30 μg/g per day, starting simultaneously with the EMC virus injection) (OB + Adipo group).[7]

HISTOLOGICAL EXAMINATION OF THE HEART

The mice were weighed and then killed by cervical dislocation on either day 4 or day 8 after viral inoculation. Cardiac tissues were immediately extracted, weighed, fixed in 10% buffered formalin and stained with haematoxylin and eosin (H&E). Two transverse sections of the ventricular myocardium were assessed for the severity of necrosis and mononuclear cell infiltration by an experienced pathologist who had no knowledge of the study design. Sections were scored according to the following scale: 1, lesions involving < 25% of the ventricular myocardium; 2, lesions involving 25 – 50% of the myocardium; 3, lesions involving 50 – 75% of the myocardium; and 4, lesions involving > 75% of the myocardium. The sections were also stained for myosin in order to confirm the presence of myocyte necrosis.

Five high-power fields (HPFs) (magnification × 400) were randomly selected from each transverse section of the myocardium, and the number of infiltrating cells counted. The number of apoptotic cells per section in these HPFs was also determined using *in situ*

terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labelling, as described previously.[8]

IMMUNOREACTIVITY OF ADIPONECTIN RECEPTORS IN MYOCYTES

To examine the immunoreactivity of adiponectin receptors 1 and 2 (AdipoR1 and AdipoR2), immunohistochemical staining using a streptavidin biotin complex method (K0675 and E0353, DAKO Cytomation Co. Ltd, Kyoto, Japan) was performed on serial sections of transverse ventricular myocardium from different mice on day 4 or day 8 after viral inoculation. The immunoreactivity of AdipoR1 and AdipoR2 in vessels and macrophages from a normal wild-type mouse that had not received viral inoculation or adiponectin administration was used as a positive control.

Rabbit polyclonal anti-mouse AdipoR1 and AdipoR2 antibodies (ADIPOR11-A and ADIPOR21-A. Alpha Diagnostic International Inc., San Antonio, TX, USA) were applied at a dilution of 1:50. Control slides were treated with normal diluted rabbit serum.

The slides were blindly reviewed by the same pathologist. The degree of adiponectin receptor reactivity was assessed in 30 randomly selected myocytes corresponding to surviving cells found on the respective H&E and myosin-stained slides, and was semi-quantitatively graded according to the degree of immunoreactivity: 0, absence of staining; 1+, weak staining; 2+, moderate staining; and 3+, strong staining.[9] The slides were also compared with the respective control slides to exclude non-specific staining.

STATISTICAL ANALYSIS

Data were expressed as the mean ± SD. Analysis of variance was used to evaluate differences in body and cardiac weights, cardiac histological scores, numbers of infiltrating or apoptotic cells in the myocardium and immunoreactivity of adiponectin receptors in myocytes between the groups. A P-value < 0.05 was considered to be statistically significant.

Results

Eight wild-type and 28 leptin-deficient mice were inoculated with EMC virus. Eighteen of the leptin-deficient mice did not receive interventional therapy (OB group); the remaining 10 leptin-deficient mice received daily injections of adiponectin (OB + Adipo group).

Eight of the mice in the OB group died from viral myocarditis during the study protocol; there were no deaths in the wild-type (WT) or OB + Adipo groups over the same period. The numbers of mice killed on day 4 and day 8 after viral inoculation were four and four, respectively, in the WT group, six and four, respectively, in the OB group, and five and five, respectively, in the OB + Adipo group.

BODY AND CARDIAC WEIGHTS

Body weights on days 0, 4 and 8 after viral inoculation were significantly higher ($P < 0.05$) in the OB and OB + Adipo groups than in the WT group (Table 1). Cardiac weights in the OB mice on day 8 after viral inoculation were significantly increased ($P < 0.05$) compared with those in the WT mice (Table 1). There was no significant difference in cardiac weight between the OB + Adipo group and the WT group.

HISTOLOGICAL FINDINGS

The histological scores of myocardial necrosis, and the numbers of infiltrating and apoptotic cells per HPF, in hearts from the different types of mice on day 4 and day 8 after viral inoculation are shown in Fig. 1. Hearts from the OB group showed severe myocardial necrosis and mononuclear cell

TABLE 1:
Body weight and cardiac weight after viral inoculation in various types of mice

	Body weight (g)			Cardiac weight (mg)	
Type	Day 0	Day 4	Day 8	Day 4	Day 8
WT	18.3 ± 1.5	18.7 ± 1.8	19.1 ± 1.5	96 ± 8	102 ± 10
OB	37.5 ± 2.4*	37.8 ± 2.9*	38.4 ± 3.1*	103 ± 7	121 ± 9*
OB + Adipo	37.8 ± 2.2*	37.3 ± 2.8*	35.6 ± 3.9*	94 ± 6	98 ± 12

WT, wild-type mice; OB, leptin-deficient ob/ob mice; OB + Adipo, OB mice receiving adiponectin.
Data are the mean ± SD.
*$P < 0.05$ compared with WT mice.

infiltration. The histological scores, numbers of infiltrating cells and numbers of apoptotic cells were significantly higher ($P < 0.05$ for all) in the OB group than in the WT group on day 8 (Fig. 1). There were no significant differences in the histological scores or the number of infiltrating or apoptotic cells between the OB + Adipo group and the WT group.

IMMUNOREACTIVITY OF ADIPONECTIN RECEPTORS

In the normal WT group, immunoreactivity for AdipoR1 was found in the arterial walls and immunoreactivity for AdipoR2 was found in macrophages. The degrees of immunoreactivity for AdipoR1 and AdipoR2 in myocytes from different mice on day 4 and day 8 are shown in Fig. 2. In the OB group, the AdipoR1 reactivity in myocytes was significantly reduced ($P < 0.05$) compared with reactivity observed in the WT group on day 4 and day 8 (Fig. 2A), but the AdipoR1 reactivity was similar in the OB + Adipo group and the WT group. There were no significant differences in the immunoreactivity for AdipoR2 among the groups (Fig. 2B).

Discussion

Circulating levels of leptin and adiponectin are either undetectable or decreased in OB mice.[10] The protective role of adiponectin against fatty liver diseases has recently been demonstrated in non-alcoholic OB mice with insulin resistance and dyslipidaemia.[11] Replacement therapy with adiponectin could in part compensate for the absence of leptin in terms of ameliorating hepatomegaly and steatosis and decreasing serum alanine aminotransferase levels,[11] although such therapy would not alter the primary aetiology. Injection of adiponectin has also been reported to elevate insulin sensitivity and alleviate hyperlipidaemia.[11] Consistent with these findings, adiponectin administration to OB mice receiving EMC viral inoculation in the present study was found to protect the OB mice from inflammatory myocardial damage.

Complementary DNA encoding the two adiponectin receptors AdipoR1 and AdipoR2, which are distantly related to the family of seven-transmembrane spanning G protein-coupled receptors, has been cloned.[12] AdipoR1 and AdipoR2 are expressed ubiquitously in most organs, with AdipoR1 being especially expressed in skeletal muscle and AdipoR2 in liver.[12] Pancreatic β-cells have also been shown to express adiponectin receptors in a cell culture system.[13] These receptors have seven transmembrane domains and activate signalling molecules

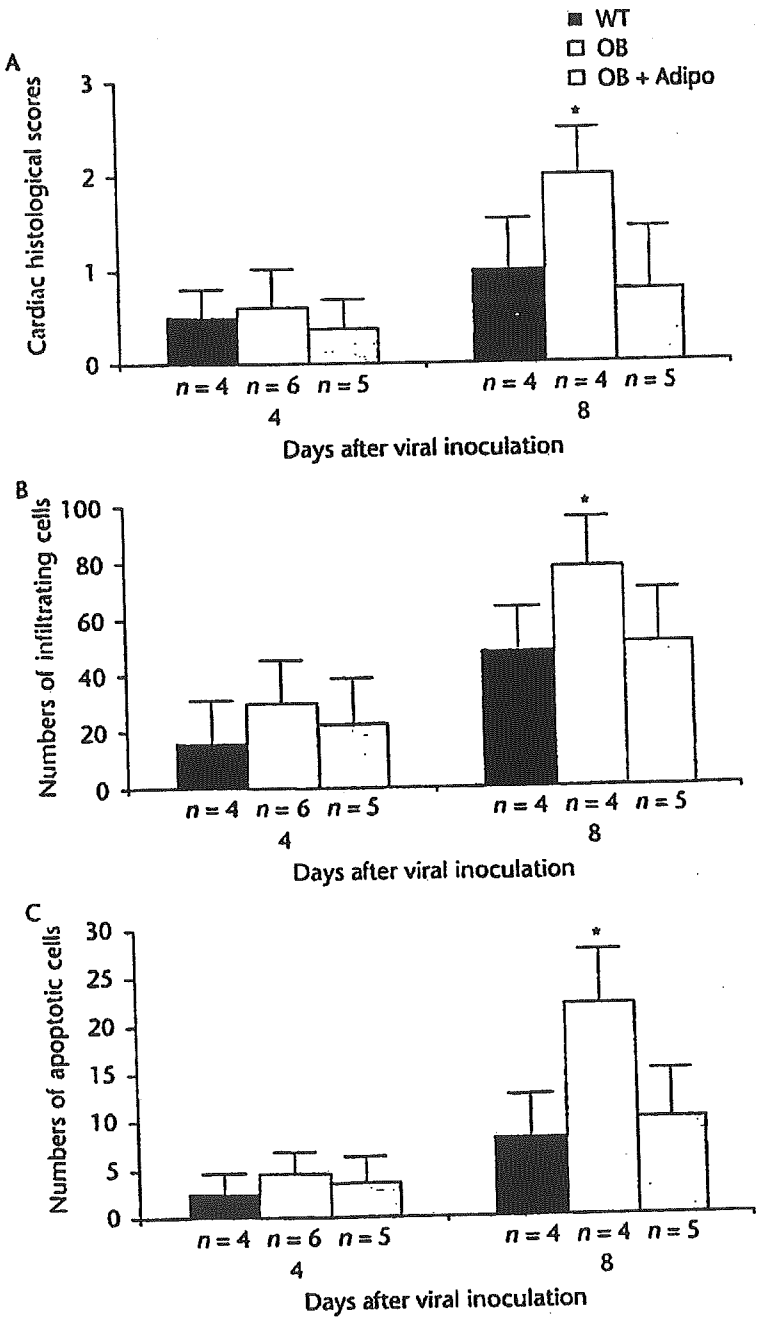

FIGURE 1: Results of histological examination of myocardial sections. (A) Histological scores, (B) numbers of infiltrating cells and (C) numbers of apoptotic cells in different types of mice on day 4 and day 8 after viral inoculation. Data are expressed as the mean ± SD. WT, wild-type mice; OB, leptin-deficient ob/ob mice; OB + Adipo, OB mice receiving subcutaneous administration of mouse adiponectin (30 μg/g daily, starting simultaneously with viral inoculation). *$P < 0.05$ compared with WT group

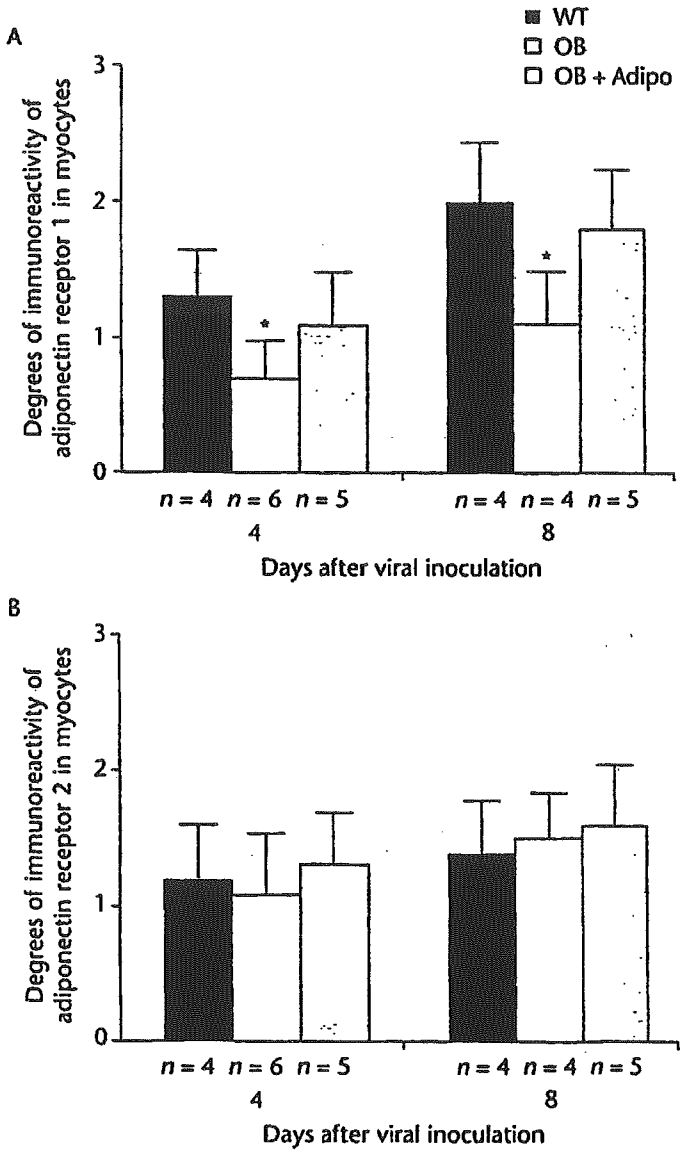

FIGURE 2: Degrees of immunoreactivity of adiponectin receptor 1 (A) or adiponectin receptor 2 (B) in myocytes from different types of mice on day 4 and day 8 after viral inoculation. Data are expressed as the mean ± SD. WT, wild-type mice; OB, leptin-deficient *ob/ob* mice; OB + Adipo, OB mice receiving subcutaneous administration of mouse adiponectin (30 μg/g daily, starting simultaneously with viral inoculation). *$P < 0.05$ compared with WT group

such as peroxisome proliferator-activated receptor-α, adenosine monophosphate-activated protein kinase, and mitogen-activated protein kinase.[12] Possible alterations to adiponectin utilization in the coronary artery and/or heart have been described in type 2 diabetic patients compared with non-diabetic patients based on the transcardiac gradient of adiponectin levels from aortic root to coronary sinus.[14]

One mechanism of impaired transcardiac utilization of adiponectin in subjects with diabetes seems to be a decreased receptor-binding ability of adiponectin in the cardiac myocytes.[14] Interestingly, in the present study we found decreased AdipoR1 immunoreactivity in damaged myocytes from OB mice with viral myocarditis, and adiponectin replacement therapy in OB mice led to recovery of the suppressed AdipoR1 reactivity. These results indicate that adiponectin may act through binding to the AdipoR1, leading to protection against the progression of myocardial inflammation.

In summary, we determined the effects of adiponectin replacement therapy on myocardial damage in OB mice with viral myocarditis. There was significantly decreased reactivity of AdipoR1 in damaged myocytes from OB mice on day 4 and day 8 after viral inoculation compared with that in myocytes from WT mice, together with elevated cardiac weights and severe inflammatory myocardial damage. Replacement of adiponectin in the OB mice inhibited the development of severe myocarditis through augmentation of the AdipoR1 reactivity in the injured myocytes. Our data suggest that exogenously administered adiponectin may inhibit the progression of viral myocarditis through binding to the AdipoR1 in leptin-deficient conditions.

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Conflicts of interest

No conflicts of interest were declared in relation to this article.

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References

1 Makris TK, Stavroulakis GA, Krespi PG, Hatzizacharias AN, Kyriaki DK, Chronakis EV, *et al*: Elevated plasma immunoreactive leptin levels preexist in healthy offspring of patients with essential hypertension. *Am Heart J* 1999; 138: 922 – 925.
2 Murdoch DR, Rooney E, Dargie HJ, Shapiro D, Morton JJ, McMurray JJ: Inappropriately low plasma leptin concentration in the cachexia associated with chronic heart failure. *Heart* 1999; 82: 352 – 356.
3 Kanda T, Takahashi T, Kudo S, Takeda T, Tsugawa H, Takekoshi N: Leptin deficiency enhances myocardial necrosis and lethality in a murine model of viral myocarditis. *Life Sci* 2004; 75: 1435 – 1447.
4 Hu E, Liang P, Spiegelman BM: *AdipoQ* is a novel adipose-specific gene dysregulated in obesity. *J Biol Chem* 1996; 271: 10697 – 10703.
5 Yamauchi T, Kamon J, Waki H, Imai Y, Shimozawa N, Hioki K, *et al*: Globular adiponectin protected *ob/ob* mice from diabetes and ApoE-deficient mice from atherosclerosis. *J Biol Chem* 2003; 278: 2461 – 2468.
6 Saito K, Tobe T, Minoshima S, Asakawa S, Sumiya J, Yoda M, *et al*: Organization of the gene for gelatin-binding protein (GBP28). *Gene* 1999; 229: 67 – 73.
7 Berg AH, Combs TP, Du X, Brownlee M, Scherer PE: The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nat Med* 2001; 7: 947 – 953.
8 Kanda T, Koike H, Arai M, Wilson JE, Carthy CM, Yang D, *et al*: Increased severity of viral myocarditis in mice lacking lymphocyte maturation. *Int J Cardiol* 1999; 68: 13 – 22.
9 Kanazawa K, Kawashima S, Mikami S, Miwa Y, Hirata K, Suematsu M, *et al*: Endothelial constitutive nitric oxide synthase protein and mRNA increased in rabbit atherosclerotic aorta despite impaired endothelium-dependent

vascular relaxation. *Am J Pathol* 1996; 148: 1949 – 1956.
10 Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, *et al*: The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 2001; 7: 941 – 946.
11 Xu A, Wang Y, Keshaw H, Xu LY, Lam KS, Cooper GJ: The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. *J Clin Invest* 2003; 112: 91 – 100.
12 Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, *et al*: Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature* 2003; 423: 762 – 769.
13 Kharroubi I, Rasschaert J, Eizirik DL, Cnop M: Expression of adiponectin receptors in pancreatic beta cells. *Biochem Biophys Res Commun* 2003; 312: 1118 – 1122.
14 Furuhashi M, Ura N, Moniwa N, Shinshi Y, Kouzu H, Nishihara M, *et al*: Possible impairment of transcardiac utilization of adiponectin in patients with type 2 diabetes. *Diabetes Care* 2004; 27: 2217 – 2221.

Address for correspondence
Dr T Kanda
Department of General Medicine, Kanazawa Medical University, 1-1 Daigaku, Uchinada-machi, Kahoku-gun, Ishikawa, 920-0293, Japan.
E-mail: kandat@kanazawa-med.ac.jp

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Adiponectin, T-cadherin and Tumour Necrosis Factor-α in Damaged Cardiomyocytes from Autopsy Specimens

T TAKAHASHI[1], S SAEGUSA[1], H SUMINO[4], T NAKAHASHI[2], K IWAI[2], S MORIMOTO[2], T NOJIMA[3] AND T KANDA[1]

[1]Department of General Medicine, [2]Department of Geriatric Medicine and [3]Department of Clinical Pathology, Kanazawa Medical University, Ishikawa, Japan; [4]Second Department of Internal Medicine, Gunma University School of Medicine, Gunma, Japan

This study determined the presence of adiponectin, T-cadherin (an adiponectin receptor) and tumour necrosis factor-α (TNF-α) in damaged myocytes from autopsied patients with acute or old myocardial infarction (MI) or dilated cardiomyopathy (DCM), using immunohistochemical staining. The enrolled patients included eight with acute MI, six with old MI and seven with DCM. Four autopsied individuals with no cardiac lesions were also enrolled as controls. Adiponectin and TNF-α were not observed in normal myocytes from control subjects, but T-cadherin was weakly detected. Immunoreactivity for adiponectin and T-cadherin was observed at the periphery of damaged myocytes from MI and DCM patients; intracellular reactivity for TNF-α was also seen. There were no statistically significant differences in the degree of reactivity for each molecule in the myocytes between the MI and DCM patients. These results suggest that the presence of adiponectin and TNF-α in damaged myocytes may contribute to the processes of myocardial injury occurring in MI and DCM.

KEY WORDS: MYOCARDIAL INFARCTION; DILATED CARDIOMYOPATHY; ADIPONECTIN; T-CADHERIN; TUMOUR NECROSIS FACTOR-α

Introduction

Adiponectin, which is also known as adipocyte complement-related protein of 30 kDa,[1] is a hormone secreted by adipocytes that acts as an anti-diabetic and anti-atherogenic cytokine.[2] It has structural homology to the protein C1q, and is found in the serum as three distinct oligomers: a trimer, a hexamer and a high molecular weight (HMW) species.[3] Concentrations of adiponectin in blood are decreased in obesity, insulin resistance and type II diabetes.[1] Adiponectin administration has been reported to lower glucose and improve insulin resistance in mice,[4] whereas adiponectin-deficient mice develop insulin resistance and diabetes.[5] This effect of adiponectin appears to be mediated by an elevation in fatty acid oxidation through activation of adenosine monophosphate-activated protein kinase[6] and peroxisome proliferator-activated receptor-α.[2]

Cadherins comprise a large family of cell-surface proteins involved in calcium-mediated cell–cell interactions and signalling. T-cadherin was initially described in the central nervous system, but its tissue distribution is more widespread; the highest expression is found in the cardiovascular system, with low levels in muscle. In the vasculature, T-cadherin is localized to the intima and media and is expressed on endothelial and smooth muscle cells. Expression was shown to be upregulated in the neointima of mouse carotid artery after injury caused by a balloon catheter.[7] Interestingly, T-cadherin has recently been reported to be a receptor for the hexameric and HMW forms of adiponectin; this was demonstrated using a series of expression-cloning studies with panned infected cells on recombinant adiponectin linked to magnetic beads.[8]

Heart failure is generally considered to begin with myocyte damage caused by a variety of pathological conditions, including ischaemia, toxins and myocardial infection. The heart compensates by dilatation and cellular hypertrophy, and eventually decompensates, leading to heart failure. The pro-inflammatory cytokine tumour necrosis factor-α (TNF-α) has been postulated to be one of the pathogenetic factors responsible for the progression from compensated to decompensated heart failure.[9] Yokoyama and colleagues[10] demonstrated that the non-failing human heart does not express TNF-α, whereas the failing human heart expresses significant amounts of this cytokine. Moreover, TNF-α immediately inhibits contractility of isolated cardiac myocytes in a dose-dependent manner; this negative inotropic action is completely reversible upon removal of TNF-α.[10]

In the light of these different findings, we hypothesized that adiponectin, its receptor T-cadherin and TNF-α may contribute to the processes of myocardial injury. In this study, the presence or absence of adiponectin, T-cadherin and TNF-α in damaged myocytes obtained from autopsied patients with acute or old myocardial infarction (MI) or dilated cardiomyopathy (DCM) was determined using immunohistochemical staining. In addition, we analysed differences in the degree of reactivity for each molecule in the myocardium between the two groups.

Patients and methods

PATIENTS

Patients with a confirmed histopathological diagnosis of acute or old MI or a diagnosis of DCM, in whom autopsy examinations were performed in the Department of Clinical Pathology, Kanazawa Medical University Hospital, Ishikawa, Japan, between 1984 and 2004, were randomly selected for inclusion in the study. Autopsied cases from the same period with no cardiac lesions of any kind were also enrolled as normal controls. All individuals were autopsied within 6 h of death. Ethical approval from our institution was not needed, since written consent for each autopsy examination was obtained from each patient's family members.

PREPARATION OF SPECIMENS

In the controls, normal myocardial tissue and surrounding pericardial tissue were dissected from the left ventricle and ventricular septum. In individuals with MI or DCM, the myocardial lesion and surrounding pericardium were dissected in the same manner. Specimens were fixed with 10% neutral buffered formaldehyde and embedded in paraffin, and thin sections were treated with haematoxylin and eosin and Azan–Mallory staining. Based on the histopathological findings, each MI lesion was staged as follows: stage I, early MI; stage II, established myocardial necrosis; stage III, macrophage infiltration; stage IV, granulation formation; stage V, scar formation.[11]

IMMUNOHISTOCHEMICAL STAINING

Immunohistochemical staining was performed on subserial transverse ventricular myocardium and pericardium paraffin sections, using a streptavidin biotin complex method (K0675 or E0466, Dako Cytomation Co. Ltd, Kyoto, Japan). The following primary antibodies were used: rabbit polyclonal anti-human adiponectin antibody at a dilution of 1:500 (AB3784P, Chemicon International Inc., Temecula, CA, USA); rabbit polyclonal anti-human T-cadherin antibody at a dilution of 1:200 (sc-7940, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA); and goat polyclonal anti-human TNF-α antibody at a dilution of 1:500 (RC210, Dako Cytomation). The immunostaining was visualized by treating the slides with 3,3'-diaminobezidine tetrahydrochloride and counterstaining with haematoxylin. Negative control slides were treated with normal diluted rabbit or goat serum. For each slide, an area containing approximately 50 myocytes corresponding to the damaged areas found on haematoxylin and eosin and Azan–Mallory staining was blindly reviewed by a pathologist and semiquantitatively graded according to the degree of immunoreactivity for adiponectin, T-cadherin and TNF-α: 0, no staining; 1+, focal staining; 2+, diffuse weak staining; 3+, diffuse moderate staining; 4+, diffuse strong staining.[12] The slides were also compared with the respective negative control slides to exclude non-specific staining.

STATISTICAL ANALYSIS

Differences in the degree of immunoreactivity for each molecule in the damaged myocytes between the MI and the DCM group were analysed using the Mann–Whitney *U*-test. A *P*-value of < 0.05 was considered to be statistically significant.

Results

PATIENT CHARACTERISTICS

Fourteen patients with a confirmed histopathological diagnosis of acute ($n = 8$) or old ($n = 6$) MI, seven patients with DCM and four controls were included in the study. Of the 14 patients with MI, nine were male and five were female. On histopathological examination, two were stage I, three were stage II, three were stage III, four were stage IV and two were stage V. The mean age of the MI patients was 74.9 ± 14.1 years (range 36 – 88 years). Of the seven patients with DCM, five were male and two were female, and the mean age was 51.4 ± 24.5 years (range 17 – 76 years). Of the four control patients without cardiac lesions, three were male and one was female, and the mean age was 55.0 ± 21.0 years (range 33 – 78 years). The main histopathological diagnoses at autopsy for the control subjects were subarachnoid haemorrhage, acute leukaemia, liver cirrhosis and pancreatic cancer, respectively.

IMMUNOREACTIVITY IN NORMAL CARDIOMYOCYTES

Adiponectin and TNF-α were not seen in non-damaged myocytes obtained from the four control subjects, but positive reactivity for adiponectin was observed in pericardial adipocytes. T-cadherin was weakly detected in normal myocytes and the surrounding vessel walls.

IMMUNOREACTIVITY IN DAMAGED CARDIOMYOCYTES

Moderate to strong immunoreactivity for adiponectin was seen at the periphery of injured myocytes from MI and DCM patients (Fig. 1). There was also weak to moderate reactivity for T-cadherin at the periphery of damaged myocytes (Fig. 2). In addition, moderate to strong intracellular reactivity for TNF-α was seen in the myocytes (Fig. 3).

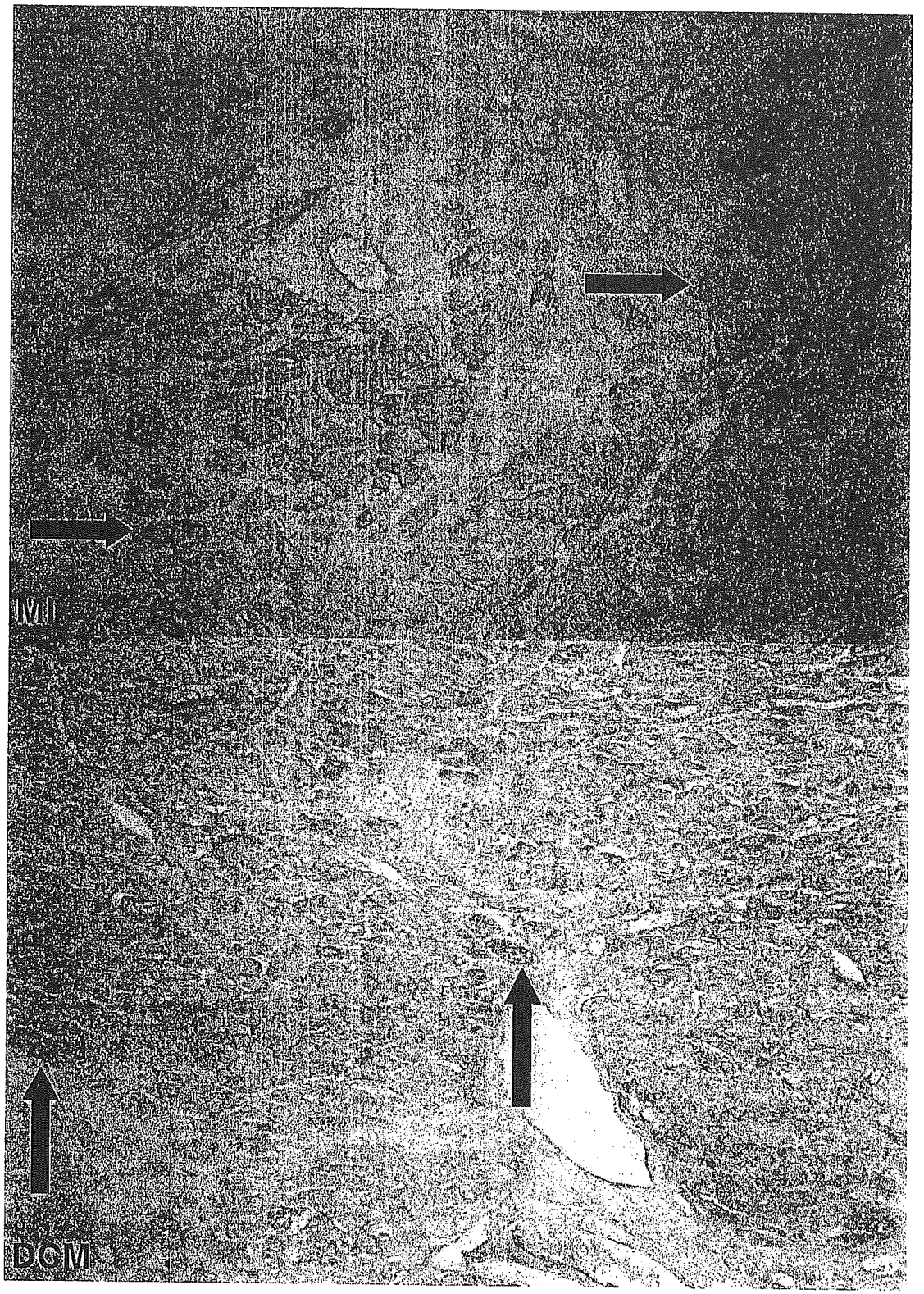

FIGURE 1: Photomicrograph showing moderate to strong adiponectin reactivity (arrows) in autopsy myocardial specimens from patients with myocardial infarction (MI) or dilated cardiomyopathy (DCM). × 75

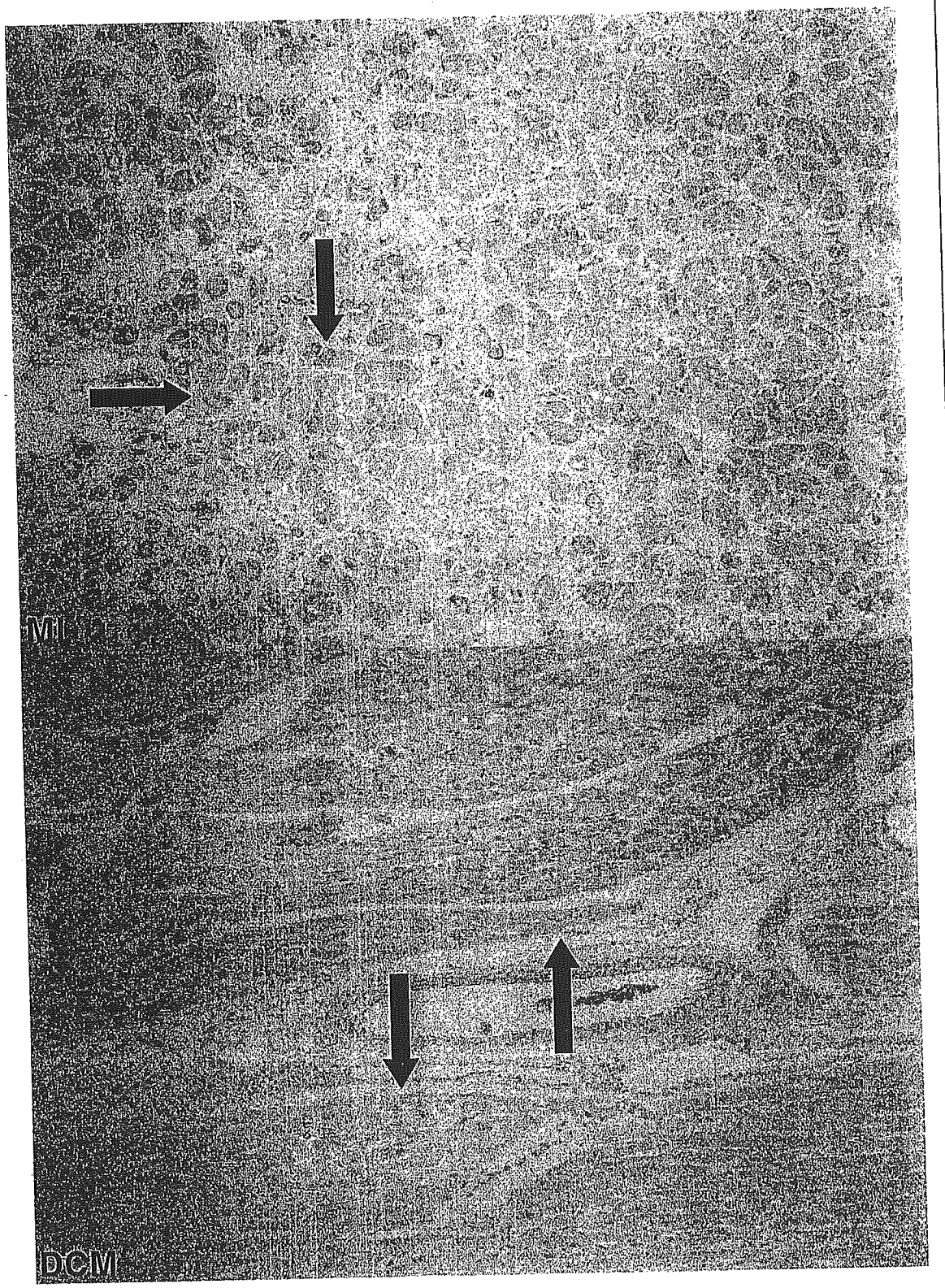

FIGURE 2: Photomicrograph showing weak to moderate T-cadherin reactivity (arrows) in autopsy myocardial specimens from patients with myocardial infarction (MI) or dilated cardiomyopathy (DCM). × 75

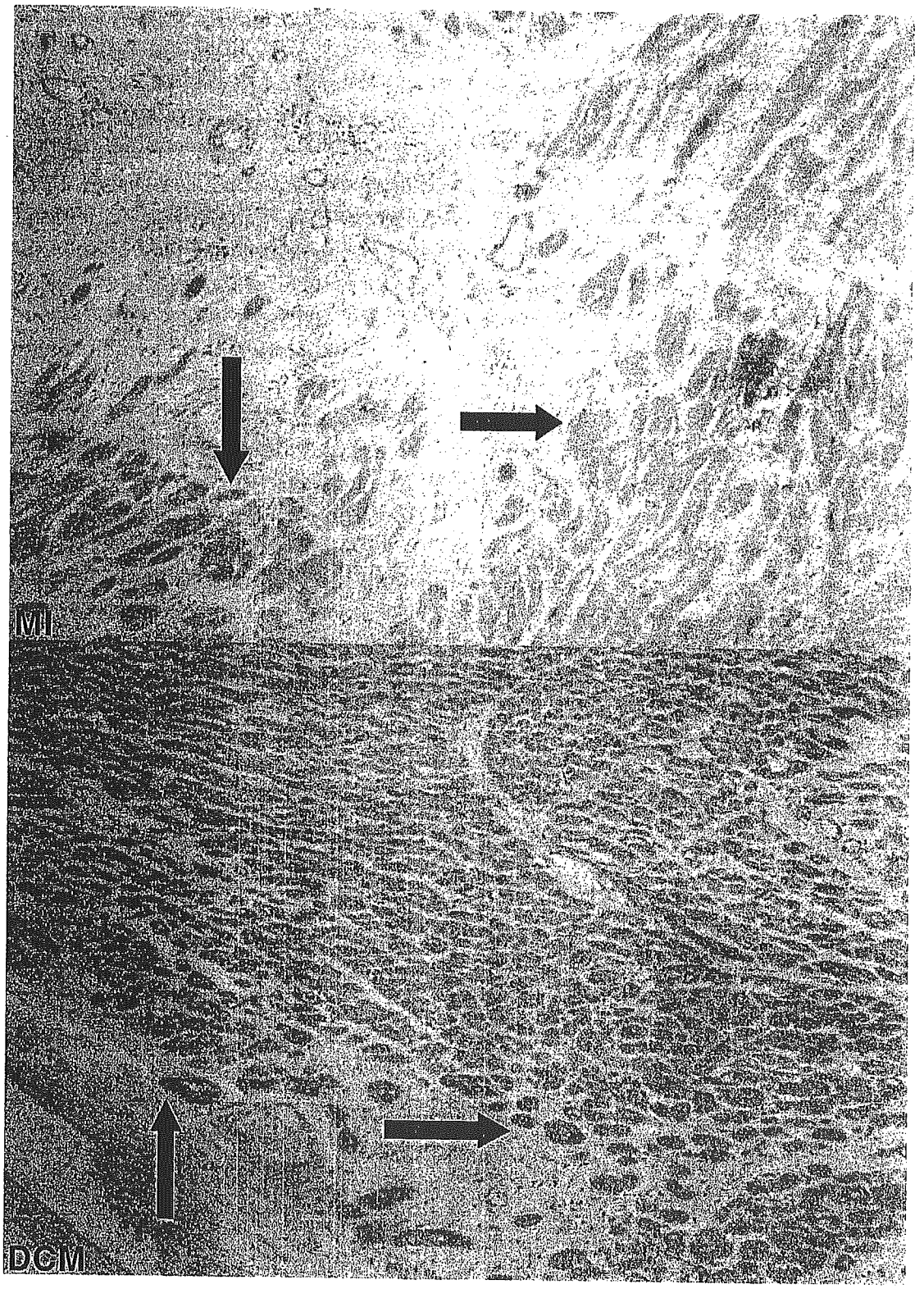

FIGURE 3: Photomicrograph showing moderate to strong intracellular tumour necrosis factor-α reactivity (arrows) in autopsy myocardial specimens from patients with myocardial infarction (MI) or dilated cardiomyopathy (DCM). × 75

The degrees of reactivity for each molecule in the damaged myocytes from individuals with MI or DCM are shown in Fig. 4. There were no statistically significant differences between the two patient groups.

Discussion

High plasma adiponectin concentrations were associated with a lower risk of MI during 6 years of follow-up in a nested case–control study among 18 225 male participants aged 40 – 75 years.[13] Possible utilization of adiponectin in the coronary artery and/or heart has been described for non-diabetic patients based on a trans-cardiac gradient of adiponectin levels from aortic root to coronary sinus.[14] It is interesting that immunostaining for adiponectin was observed at the periphery of damaged cardiomyocytes in lesions at the granulative stage obtained from autopsied hearts with infarction.[15] In another immunohistochemical analysis, the boundaries of mouse hepatocytes were positive for adiponectin after 3 – 6 h of carbon tetrachloride treatment, and the cytoplasm was intensely stained after 18 h of treatment.[16] The authors suggested that adiponectin was produced by the damaged hepatocytes, and undergoes tissue damage-induced transcriptional regulation.[16] In the present study, adiponectin was seen in damaged myocytes from both DCM and MI patients, suggesting that the adipose tissue-specific cytokine adiponectin may have important implications for the processes of myocardial damage.

The adiponectin receptors AdipoR1 and AdipoR2 are expressed ubiquitously in most organs, but in particular AdipoR1 is found in skeletal muscle and AdipoR2 in liver.[17] Complementary DNA for these receptors has been cloned, and they have been shown to be distantly related to the family of seven-

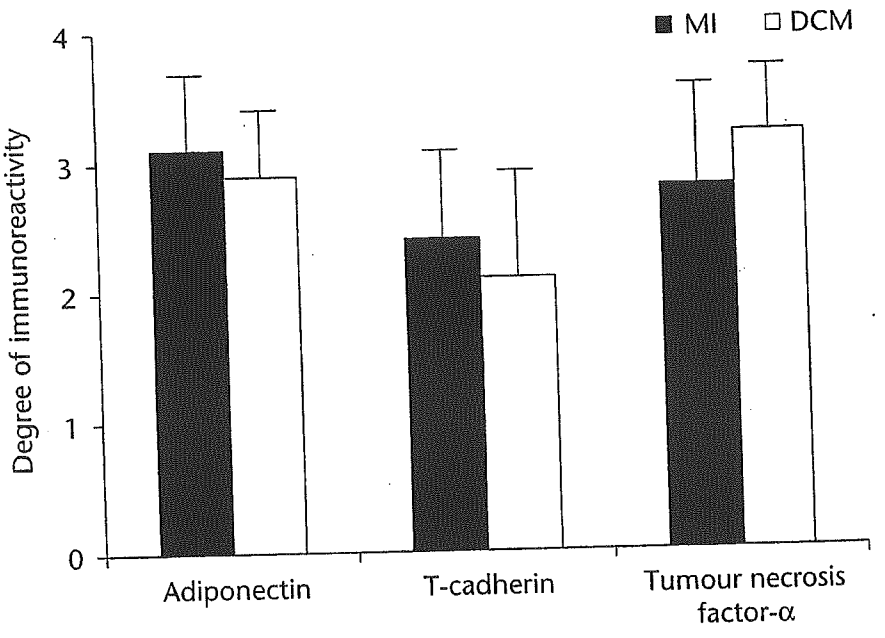

FIGURE 4: Degrees of immunoreactivity for adiponectin, T-cadherin and tumour necrosis factor-α in damaged myocytes obtained from 14 autopsied patients with myocardial infarction (MI) or dilated cardiomyopathy (DCM)

transmembrane-spanning G protein-coupled receptors.[17] However, these receptors have an inverted topology with an intracellular N terminus, unlike other seven-transmembrane spanning receptors.[8] In addition, the extracellular portion of these molecules is small, which is distinct from the members of this class of receptors that bind peptide hormone.[8] T-cadherin, a glycosylphosphatidylinositol-anchored extracellular protein, has been shown to be a novel receptor for the hexameric and HMW forms of adiponectin.[8] In the present study, both T-cadherin and adiponectin were seen in damaged myocardial cells from autopsied MI or DCM patients. This indicates that damaged cardiac cells may possess an adiponectin autocrine system, which leads to protection against the progression of myocardial injury. TNF-α expression was also observed in the damaged myocytes from subjects with DCM and MI using immunohistochemical staining as previously demonstrated.[9] We found cytoplasmic or perinuclear distribution of TNF-α expression in the damaged myocytes, and peripheral distribution of adiponectin expression in the injured cells.

In conclusion, the results of the present study suggest that the presence of adiponectin and TNF-α in damaged myocytes may contribute to the processes of myocardial injury occurring in MI and DCM.

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Conflicts of interest

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References

1 Hu E, Liang P, Spiegelman BM: AdipoQ is a novel adipose-specific gene dysregulated in obesity. *J Biol Chem* 1996; **271:** 10697 – 10703.
2 Yamauchi T, Kamon J, Waki H, Imai Y, Shimozawa N, Hioki K, *et al*: Globular adiponectin protected ob/ob mice from diabetes and ApoE-deficient mice from atherosclerosis. *J Biol Chem* 2003; **278:** 2461 – 2468.
3 Tsao TS, Tomas E, Murrey HE, Hug C, Lee DH, Ruderman NB, *et al*: Role of disulfide bonds in Acrp30/adiponectin structure and signaling specificity. Different oligomers activate different signal transduction pathways. *J Biol Chem* 2003; **278:** 50810 – 50817.
4 Berg AH, Combs TP, Du X, Brownlee M, Scherer PE: The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nat Med* 2001; **7:** 947 – 953.
5 Maeda N, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaretani H, *et al*: Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nat Med* 2002; **8:** 731 – 737.
6 Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, *et al*: Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med* 2002; **8:** 1288 – 1295.
7 Ivanov D, Philippova M, Antropova J, Gubaeva F, Iljinskaya O, Tararak E, *et al*: Expression of cell adhesion molecule T-cadherin in the human vasculature. *Histochem Cell Biol* 2001; **115:** 231 – 242.
8 Hug C, Wang J, Ahmad NS, Bogan JS, Tsao TS, Lodish HF: T-cadherin is a receptor for hexameric and high-molecular-weight forms of Acrp30/adiponectin. *Proc Natl Acad Sci USA* 2004; **101:** 10308 – 10313.
9 Torre-Amione G, Kapadia S, Lee J, Durand JB, Bies RD, Young JB, *et al*: Tumor necrosis factor-alpha and tumor necrosis factor receptors in the failing human heart. *Circulation* 1996; **93:** 704 – 711.