

## SUBJECTS AND METHODS

### *Identification of Cases of Spontaneous Fracture*

We conducted an observational study in a 1993-bed hospital for elderly subjects, serving as a hospital and a long-term care facility for the elderly, which is a common combination of medical and care services in Japan (7). Reports of accidents and/or possible abuse in the hospital were constructed from the daily observations of nursing staff, and a prospective survey was continued from January 1, 1998 to December 31, 2004. At the start of observation, the male/female ratio of inpatients was about 3/10, the mean age was 79 and 85 years old, respectively, the number of completely bedridden elderly defined as those requiring complete assistance for transfer from bed to armchair and/or stretcher was 481 (about 24% of the total inpatients), and the number of patients with joint contracture(s) in either the upper and/or lower extremities within the completely bedridden patients was 267 (about 55% of the completely bedridden elderly). The sex ratio, mean age, and numbers of completely bedridden subjects and those with joint contractures were about the same during the observational period. Spontaneous fractures were defined as fractures occurring in long-bones in completely bedridden elderly, without any apparent external force or abuse, during daily care procedures.

### *Data Collection*

The clinical background of the patients and features of spontaneous fractures occurring in long bones were reviewed from their medical records. Examined items included age,

sex, length of bedridden period, main causes for bedridden state, nutritional state evaluated by serum albumin level, complications just before the fracture, date of onset and locations of spontaneous fractures, presence/absence of particular episode causing the fracture(s), presence/absence of joint contractures, past history of fractures of the same bone and other long bones, presence/absence of hemiplegia, detailed treatment procedures, and outcome up to one year after the fracture(s).

## RESULTS

A total of 19 spontaneous fractures occurring in long bones in 18 individuals (one man and 17 women) were identified during the study period. Case No. 7 had two successive fractures, and they were included in this study (Table 1). The clinical features and outcome of the spontaneous fractures are summarized in Table 1.

### *Clinical Backgrounds of Patients with Spontaneous Fractures*

Mean ( $\pm$  standard deviation) age of the 18 patients was  $88 \pm 9$  years old (range: 63-100 years old). The mean estimated length of the bedridden period at the time of occurrence of fractures was  $7 \pm 6$  years (range: 2-22 years). The main causes for a bedridden state included multiple cerebral lacunar infarction in 8 patients, cerebral infarction in 3, senile dementia in 3, intracerebral hemorrhage in 2, and Parkinson disease and hip fracture in one each.

The mean serum albumin level was  $32 \pm 4$  g/L, and 12 (67%) of the 18 individuals showed a decreased serum albumin level (normal range: 34-49 g/L), reflecting a poor nutritional state of bedridden subjects.

As complications just before the occurrence of fracture, 4 patients had suffered from aspiration pneumonia, 3 subjects from pressure ulcers, and 2 from urinary tract infection.

### *Characteristics of Spontaneous Fractures on Long Bones*

Spontaneous fractures occurring in long bones affected characteristic sites, including the supracondylar area of the femur in 8 patients, intertrochanteric area of the femur in 2, shaft of the femur and surgical neck of the femur in one each, the surgical neck of the humerus in 2, humerus in 2, supracondylar area of the humerus in one, and the proximal phalanx of the index finger in one. Representative X-ray findings of spontaneous fractures of long bones are shown in Figure 1.

Episodes that might have caused the spontaneous fractures in long bones were identified as changing a diaper in one patient, and treating a decubitus ulcer in another. However, in the remaining 16 patients who developed fractures, the episodes were unclear. Moreover, 10 of 18 patients had previously experienced fractures of long bones that were either traumatic or non-traumatic in origin, including 6 patients with a history of fracture of the same bone, where the spontaneous fracture re-occurred. Of the 5 hemiplegic patients, 4 suffered from fractures on the paralytic side.

The most characteristic features of spontaneous fractures of long bones in completely bedridden subjects was found to be proximal and/or distal joint contracture(s) identified adjacent to the fractured bone. Joint contractures were observed at the proximal site in 17 patients, at the distal site in 16 patients, and at either proximal or distal sites of the fractured bones in all 18 patients.

### *Treatment Procedures and Prognosis for Spontaneous Fractures on Long Bones*

Of the 18 patients, 9 were treated with a plaster bandage, 5 with a splint bandage, 2 with

an adhesive plaster bandage and one with traction only. The remaining one underwent operation with open reduction and internal fixation. However, re-operation was needed 4 months later since three pieces of the compressed hip screw penetrated her knee joint (Case No. 14).

One subject died within one month after the occurrence of fracture because of worsening of aspiration pneumonia which had been present before the fracture (Case No. 1). On the other hand, 17 of the 18 patients recovered from the fractures, mainly with simple treatment with a bandage method, within approximately 2 months of the fracture. Despite healing of the fractures, one died because of gastrointestinal bleeding 3 months after the accident (Case No. 4), and 3 died because of pneumonia within one year after the fracture (Case No. 2, 5, and 7), reflecting their old age and bedridden state.

## DISCUSSION

The present study showed characteristic features of spontaneous fractures of long bones in completely bedridden elderly subjects similar to those reported previously (3-6). The patients with fractures were very old, with an average age of 88 years, and 14 (78%) of the 18 patients were aged 85 years or older. The average period of a bedridden state was 7 years, and most of them showed a poor nutritional state represented by the serum albumin level. Of the 18 patients, 2 were classified as having minimal trauma fractures and the remaining as having non-traumatic fractures, according to the criteria described by Kane and colleagues (5). Moreover, the locations of the spontaneous fractures observed in this study were also partly compatible with those in previous reports (3-6). In our study, 12 femoral fractures (63% of the total fractures) including 8 supracondylar fractures of the femur (42%), and 6 humeral fractures (32%) were the two dominant sites of the fractures. On the other hand, of 13 spontaneous insufficiency fractures of long bones in 6 individuals, the fractures affected the femoral neck in 2, the distal femoral shaft in 3, the tibia in 6, and the humerus and radius in one each (3). Sherman (4) added another elderly non-weight-bearing woman who developed a fracture of the humerus while being transferred. Among 16 subjects with impaired mobility and minimal trauma fractures in another survey, the fractures affected the non-hip lower extremity in 9, femoral neck in 4, and upper extremity in 3 (8). More recently, Martin-Hunyadi and colleagues (6) have reported 55 spontaneous long-bone insufficiency fractures affecting the femoral neck in 15, middle or distal femur in 13,

tibia and/or fibula in 14, humerus in 11, and elbow in 2 among 53 extremely elderly residents including 38 completely bedridden patients in long-term nursing homes. Spontaneous fractures might re-occur at the same location as previous fractures, as in Case No. 7, as suggested previously (3, 6). As shown in one patient with hemiparesis in a previous report (3), 4 of the 5 hemiplegic patients suffered from fractures on the paralytic side.

One of the characteristic features of spontaneous fractures in bedridden elderly in our study is that one-third of the subjects had previous fractures of the same bone where the spontaneous fractures re-occurred. Moreover, more than half of the patients had a history of fractures of long bones of traumatic or non-traumatic origin. This observation shows that elderly subjects with previous long-bone fractures during non-bedridden periods are particularly prone to reoccurrence of long-bone fractures, especially in the healed bone even after the start of their bedridden status.

As another characteristic feature in the present study, joint contractures adjacent to the fractures were found in all individuals. There were no cases of spontaneous fractures in the population of bedridden elderly without joint contractures during our survey. Joint contractures might be one of the risk factors leading to fractures. Of the 18 completely bedridden patients with fractures, joint contractures were observed at the proximal site in 17, at the distal site in 16, and at either proximal or distal sites of the fractured bones in all 18 subjects. As described by Kane et al. (5) and Martin-Hunyadi et al. (6), a marked decrease in bone mass and bone quality due to multiple risk factors such as immobilization, disease, and malnutrition should also be a fundamental factor in

fracture.

Regarding hemiplegic patients, we previously reported that bone mineral density (BMD) decreased more rapidly on the paretic side compared to the non-paretic side (9), and hemiplegic patients showed severe joint contractures on the paretic side compared to the non-paretic side in our study. Although BMD was not assessed in our patients, we speculate that the fractures occurred at the weakest point of the bone near the contracted articulation. Joint contractures of an extremity fix the limb to the torso, so that the contracted joint acts like a supporting point of leverage, so that any minimal external force or torque maneuver during passive transfer, lifting, etc. on the distal part of a long bone might easily make a bone with low BMD reach its fracture threshold. Although the direct causes of fracture were unknown in the majority of cases, we speculate that the fractures were mostly due to “transfer” or “turning” during care. A subtle external force such as changing a diaper, washing, or putting the patient in an ambulatory or sitting position might produce a deforming force strong enough to make the bone reach its fracture threshold.

Etiologically, the spontaneous fractures of long bones in our bedridden elderly could be classified into insufficiency and/or pathologic fractures (8, 10-12). A decrease in bone mass and/or bone quality due to multiple factors including pathologic or traumatic conditions may have been a fundamental risk factor in all the fractures. These may include pathologic fractures found in patients whose bones are invaded by tumor or subjected to radiotherapy (10), and spontaneous fractures of the femoral neck in elderly subjects without a history of significant trauma or an unusual increase in daily activity



(10-12). On the other hand, Miller et al. (8) reported 31 so-called spontaneous fractures which occurred in 29 institutionalized patients (mean age 20 years, range 7 to 64 years), who were primarily bedridden because of severe cerebral palsy associated with brain injury. The authors speculated multiple causes for the occurrence of spontaneous fractures in the subjects, including disuse atrophy of bones, nutritional deficiency, and severe osteomalacia resulting from anti-convulsant administration. Moreover, the patients had progressive joint contractures from cerebral palsy. These observational data are partly compatible with the high incidence of joint contractures near the spontaneous fractures observed in completely bedridden elderly in the present survey.

Contrary to our expectation, the outcomes of the spontaneous fractures of long bones were relatively good. Most of our patients recovered from the fractures mainly by conservative treatment with a bandage, within approximately 2 months of the onset. The outcome of the fractures may be compatible with that reported by Kane and Goodwin (3), in which all 6 cases healed within 2 to 4 months with a cast, brace and/or splint. On the other hand, a relatively high mortality of 24% in 53 patients was demonstrated in elderly individuals with this kind of fracture (6). Indeed, patients with severe complications, such as pneumonia, might have a poor prognosis after fracture, like the subject in our observation. It was unclear whether the occurrence of spontaneous fractures even after recovery might affect the life expectancy of individuals.

Considering the pain suffered by patients with long-bone fractures, we suggest that family members should be informed about the possibility that spontaneous fractures of long bones may occur if subjects are permanently bedridden during hospitalization. The

preventive approach is extremely limited for patients with severe joint contractures, poor nutritional state and/or hemiplegia, although there have been several reports demonstrating that supplementation with calcium and vitamin D is effective to prevent falls and consequent fractures in individuals in nursing homes (13). Treatment with bisphosphonates has also been used for bone loss in paraplegic patients (14). There are no papers regarding the effect of medication that could reduce bone-resorption or promote bone-formation in permanently bedridden patients. We also should advise nursing staff that long-term bedridden individuals are at high risk for spontaneous fractures that might occur during “transfer” and “turning” procedures. The incidence of spontaneous fractures of long bones has increased in recent years in our observation (see Table 1), especially after the introduction of the Japanese long-term care insurance system in 2000. In order to prevent fractures, new techniques for transferring and turning, and a preventive approach to joint contractures should be developed. Meanwhile, any audible “crack”, swelling, or deformity during “transfer” and “turning” procedures should not be ignored.

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**Table 1. Clinical Characteristics of Patients with Spontaneous Fractures of Long Bones.**

No.	Age (yrs)	Sex	Bedridden Period (yrs)	Main Cause of Bedridden State	Serum Albumin Level (g/L)	Complications	Date of Spontaneous Fracture	Location of Fracture	Episode That Might have Caused Fracture
1	91	Female	7	Cerebral infarction	29	Aspiration pneumonia, pressure ulcers	27-Oct-98	Shaft of right humerus	Unknown
2	91	Female	2	Multiple cerebral lacunar infarction	33	Pressure ulcers	4-Apr-99	Supracondylar area of left femur	Unknown
3	91	Female	4	Multiple cerebral lacunar infarction	33	(-)	25-May-99	Supracondylar area of right femur	A "crack" was heard during changing a diaper
4	100	Female	3	Hip fracture	33	(-)	31-May-99	Supracondylar area of right femur	Unknown
5	86	Female	20	Multiple cerebral lacunar infarction	24	Aspiration pneumonia	3-Apr-00	Supracondylar area of left femur	Unknown
6	93	Female	3	Senile dementia	27	(-)	3-Dec-00	Supracondylar area of right femur	Unknown (knee joint was found to be swollen and skin reddish when nurse was applying ointment)
7	82	Female	5	Intracerebral hemorrhage	31	Aspiration pneumonia	1-Jun-01	1) Shaft of right humerus 2) Shaft of right humerus	Unknown
8	63	Male	17	Intracerebral hemorrhage	39	(-)	8-Aug-01	Supracondylar area of left femur	Unknown
9	85	Female	10	Multiple cerebral lacunar infarction	34	Aspiration pneumonia	8-Sep-01	Intertrochanteric area of right femur	Unknown
10	89	Female	5.5	Multiple cerebral lacunar infarction	30	(-)	15-Nov-01	Surgical neck of left humerus	Unknown
11	96	Female	3.7	Cerebral infarction	25	(-)	18-Jan-02	Supracondylar area of left femur	Unknown
12	82	Female	22	Multiple cerebral lacunar infarction	35	(-)	30-Apr-02	Supracondylar area of right humerus	Unknown
13	94	Female	12	Multiple cerebral lacunar infarction	36	Urinary tract infection	9-May-02	Surgical neck of right humerus	Unknown (rigid contracted joint was found to be floppy when changing diaper)
14	73	Female	2	Parkinson disease	33	Urinary tract infection, pressure ulcers	6-Sep-03	Shaft of right femur	Fracture might have occurred while lifting her right leg during treatment of a decubitus ulcer
15	90	Female	4	Senile dementia	37	(-)	22-Dec-03	Supracondylar area of left femur	Unknown
16	89	Female	4	Senile dementia	32	(-)	30-Dec-03	Proximal phalanx of left second finger	Unknown
17	98	Female	2	Multiple cerebral lacunar infarction	32	(-)	14-Jan-04	Intertrochanteric area of right femur	Unknown
18	90	Female	6	Multiple cerebral lacunar infarction	34	(-)	16-Jul-04	Surgical neck of left femur	Unknown

History of Fracture of Same Bone	History of Other Long-bone Fracture	Side of Hemiplegia if Present	Joint Contractures Adjacent to Fractured Bone	Treatment of Fracture	Outcome
(-)	Right hip fracture by falling	Right	Shoulder and elbow	Splint bandage	Died one month later due to aspiration pneumonia Recovered (died 9.5 month later due to aspiration pneumonia)
(-)	(-)	(-)	Hip and knee	Plaster bandage	
Left hip fracture by falling	(-)	(-)	Hip and knee	Plaster bandage	Recovered
(-)	Left hip fracture by falling	(-)	Hip and knee	Plaster bandage	Recovered (died 3 month later due to gastrointestinal bleeding)
(-)	Left hip fracture by falling	(-)	Hip and knee	Plaster bandage	Recovered (died 9 month later due to aspiration pneumonia)
Right hip fracture by falling	(-)	(-)	Hip and knee	Plaster bandage	Recovered
Fracture of right humerus by falling	(-)	Right	Shoulder and elbow	Splint bandage	Recovered (died 5 month later due to aspiration pneumonia)
(-)	Left hip fracture by falling	Right	Knee	Plaster bandage	Recovered
Fracture of right humerus by falling	(-)	(-)	Hip and knee	Splint bandage	Recovered
(-)	(-)	Left	Shoulder	Splint bandage	Recovered
Left hip fracture by falling	(-)	(-)	Hip and knee	Plaster bandage	Recovered
(-)	(-)	Right	Shoulder and elbow	Adhesive plaster bandage	Recovered
(-)	(-)	(-)	Shoulder and elbow	Splint bandage	Recovered
(-)	(-)	(-)	Hip and knee	Open reduction and internal fixation	Recovered
Left hip fracture by falling	Right hip fracture by falling	(-)	Hip and knee	Plaster bandage	Recovered
(-)	(-)	(-)	Fingers and wrist	Adhesive plaster bandage	Recovered
(-)	(-)	(-)	Hip and knee	Traction only	Recovered
(-)	(-)	(-)	Hip and knee	Plaster bandage	Recovered

# Adiponectin, T-cadherin and Tumour Necrosis Factor- $\alpha$ in Damaged Cardiomyocytes from Autopsy Specimens

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This study determined the presence of adiponectin, T-cadherin (an adiponectin receptor) and tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) 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- $\alpha$  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- $\alpha$  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- $\alpha$  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- $\alpha$**

## Introduction

Adiponectin, which is also known as adipocyte complement-related protein of 30 kDa,<sup>1</sup> is a hormone secreted by adipocytes that acts as an anti-diabetic and anti-atherogenic cytokine.<sup>2</sup> 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.<sup>3</sup> Concentrations of adiponectin in blood are

decreased in obesity, insulin resistance and type II diabetes.<sup>1</sup> Adiponectin administration has been reported to lower glucose and improve insulin resistance in mice,<sup>4</sup> whereas adiponectin-deficient mice develop insulin resistance and diabetes.<sup>5</sup> This effect of adiponectin appears to be mediated by an elevation in fatty acid oxidation through activation of adenosine monophosphate-activated protein kinase<sup>6</sup> and peroxisome proliferator-activated receptor- $\alpha$ .<sup>2</sup>

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.<sup>7</sup> 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.<sup>8</sup>

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- $\alpha$  (TNF- $\alpha$ ) has been postulated to be one of the pathogenetic factors responsible for the progression from compensated to decompensated heart failure.<sup>9</sup> Yokoyama and colleagues<sup>10</sup> demonstrated that the non-failing human heart does not express TNF- $\alpha$ , whereas the failing human heart expresses significant amounts of this cytokine. Moreover, TNF- $\alpha$  immediately inhibits contractility of isolated cardiac myocytes in a dose-dependent manner; this negative inotropic action is completely reversible upon removal of TNF- $\alpha$ .<sup>10</sup>

In the light of these different findings, we hypothesized that adiponectin, its receptor T-cadherin and TNF- $\alpha$  may contribute to the processes of myocardial injury. In this study, the presence or absence of adiponectin,

T-cadherin and TNF- $\alpha$  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.<sup>11</sup>

### 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- $\alpha$  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- $\alpha$ : 0, no staining; 1+, focal staining; 2+, diffuse weak staining; 3+, diffuse moderate staining; 4+, diffuse strong staining.<sup>12</sup> 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  $74.9 \pm 14.1$  years (range 36 – 88 years). Of the seven patients with DCM, five were male and two were female, and the mean age was  $51.4 \pm 24.5$  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  $55.0 \pm 21.0$  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- $\alpha$  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- $\alpha$  was seen in the myocytes (Fig. 3).



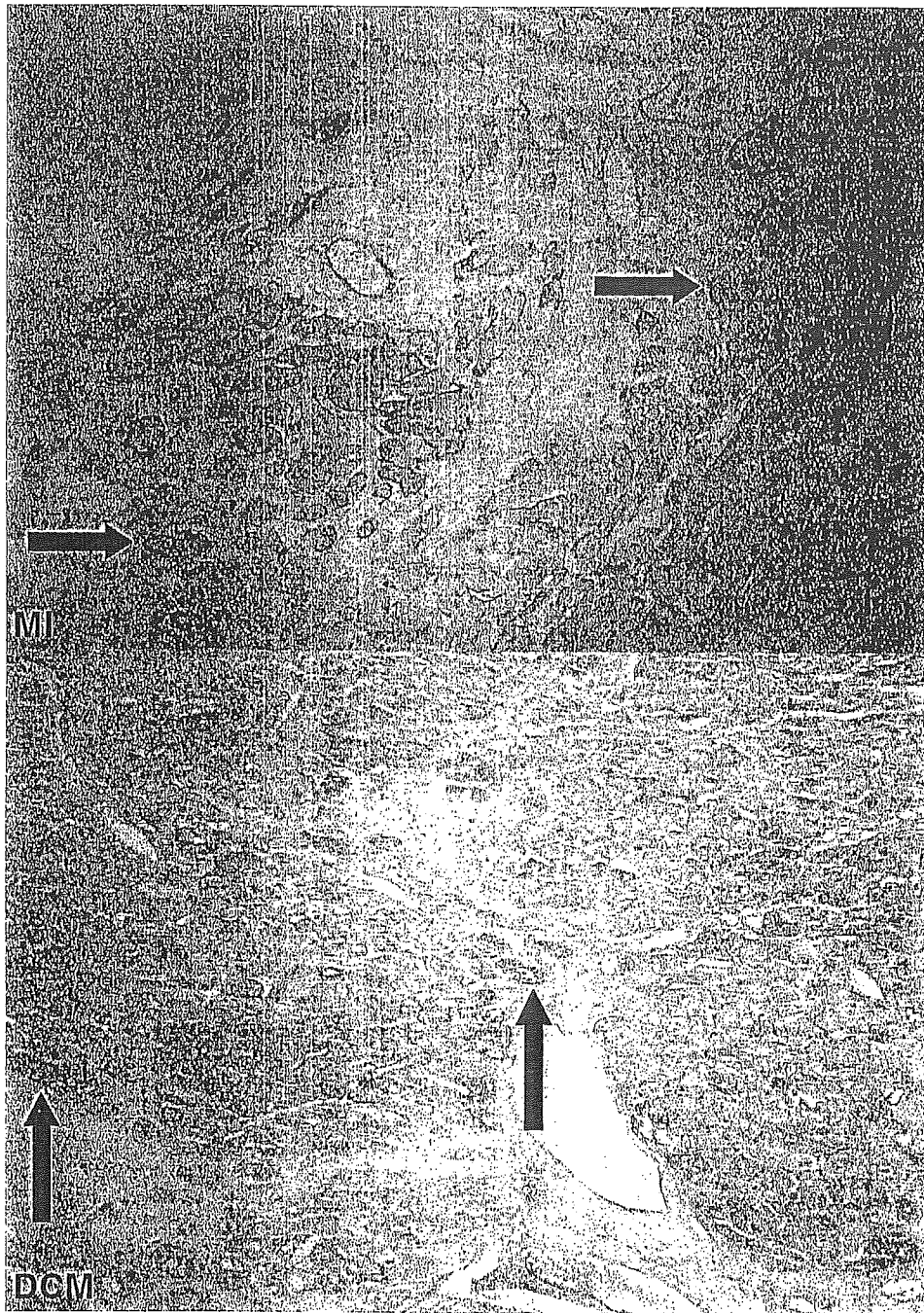


FIGURE 1: Photomicrograph showing moderate to strong adiponectin reactivity (arrows) in autopsy myocardial specimens from patients with myocardial infarction (MI) or dilated cardiomyopathy (DCM).  $\times 75$

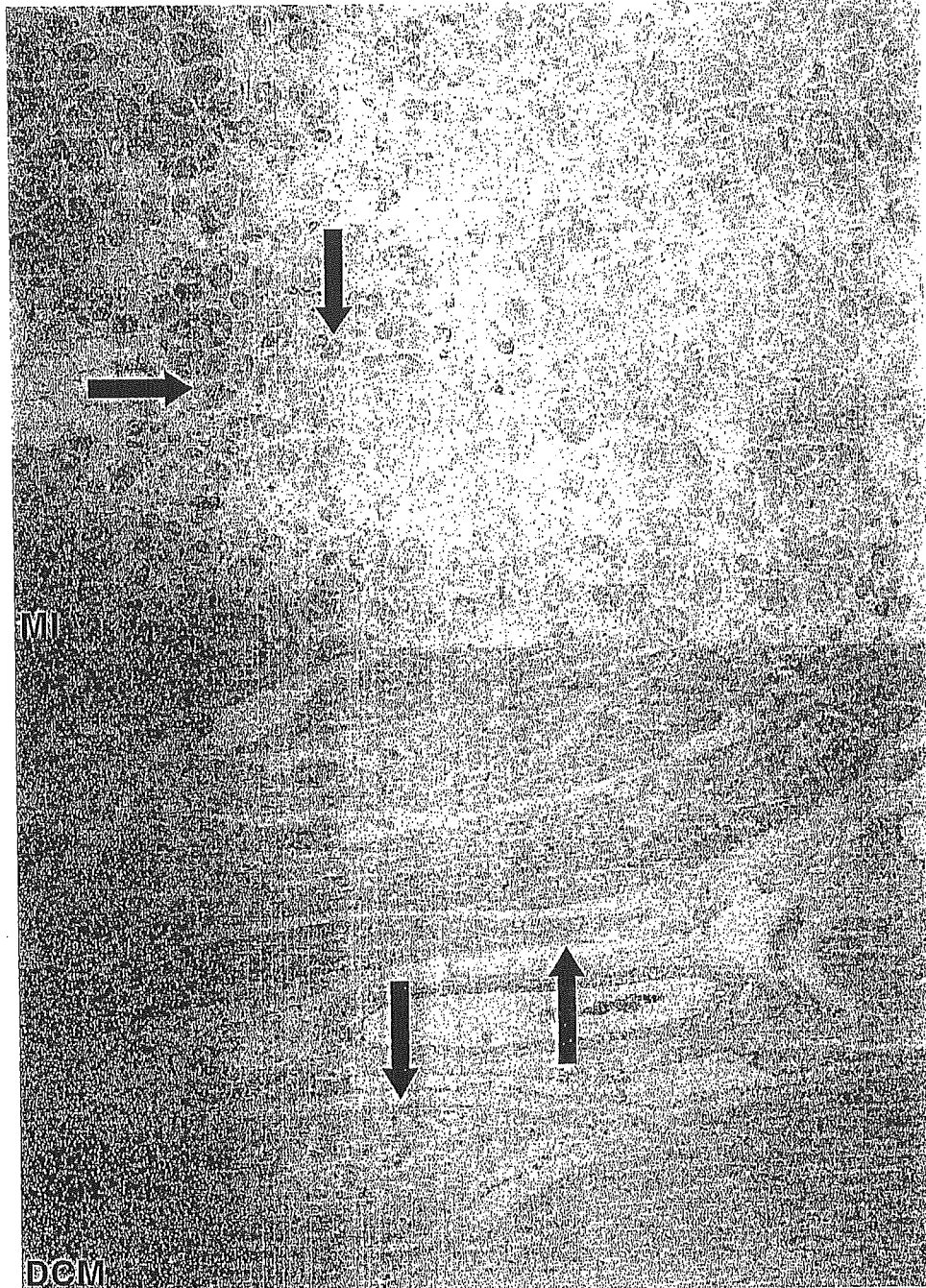


FIGURE 2: Photomicrograph showing weak to moderate T-cadherin reactivity (arrows) in autopsy myocardial specimens from patients with myocardial infarction (MI) or dilated cardiomyopathy (DCM).  $\times 75$

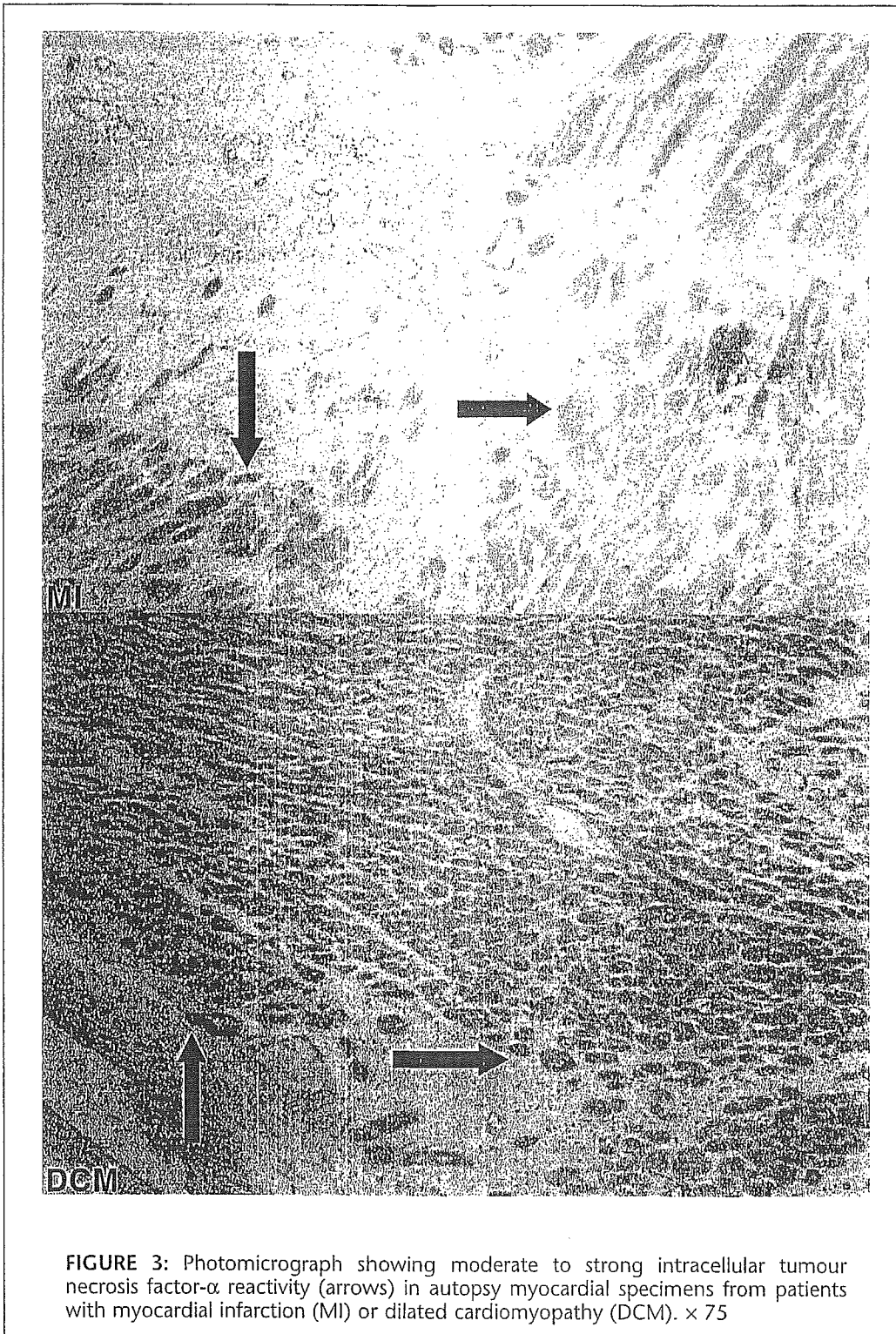


FIGURE 3: Photomicrograph showing moderate to strong intracellular tumour necrosis factor- $\alpha$  reactivity (arrows) in autopsy myocardial specimens from patients with myocardial infarction (MI) or dilated cardiomyopathy (DCM).  $\times 75$

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.<sup>13</sup> Possible utilization of adiponectin in the coronary artery and/or heart has been described for non-diabetic patients based on a transcardiac gradient of adiponectin levels from aortic root to coronary sinus.<sup>14</sup> 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.<sup>15</sup> 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.<sup>16</sup> The authors suggested that adiponectin was produced by the damaged hepatocytes, and undergoes tissue damage-induced transcriptional regulation.<sup>16</sup> 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.<sup>17</sup> Complementary DNA for these receptors has been cloned, and they have been shown to be distantly related to the family of seven-

