Virchows Arch (1999) 434:159–165

ORIGINAL ARTICLE

Masayo Oumi · Torao Yamamoto

Ultrastructural changes in myocardial cells of rats fed a low protein diet

Received: 22 April 1998 / Accepted: 7 September 1998

Abstract Ultrastructural changes in the ventricular myocardial cells in rats fed a low protein diet were examined by electron microscopy. The most striking changes were observed in the I-band region of the sarcomeres, which occurred very occasionally in myofibrils. In the sarcomere affected the I-band region was often fractured and/or disintegrated on one side, leaving an extended space, while the opposing I-band region disappeared along with dislocation of the intact A-band toward the adjacent Z-line. This dislocation was presumably attributed to the elasticity of titins connecting between the end of thick filaments and the Z-line. Fractured I-band regions were often accompanied by the dilated sarcoplasmic reticulum in the close vicinity of them. In some myofibrils the streaming and/or disruption of the Z-line were occasionally observed where disarrangement of thick and thin myofilaments were usually present. The study suggests that the fracture of the I-band region, consisting of actin and titin filaments, and the streaming of the Z-line of myofibrils are due to a proteolytic action of calpain and/or cathepsin L, which are activated by leaked Ca^{2+} ion and/or by modification of internal circumstances of the cytoplasm induced by a low protein diet, thus resulting in a low cardiac output.

Key words Myocardial cells · Protein malnutrition · Ultrastructural changes · I-band fracture · Streaming of Z-line

Introduction

Recently malnutrition, particularly protein deficiency, has again become a serious problem in refugee camps, and in densely populated and underdeveloped areas. Even in modern hospitals, physicians have to pay attention to protein malnutrition, which is often encountered during hospitalization of patients. It is well known that such forms of protein malnutrition as kwashiorkor can affect the structure and function of the heart [1, 2, 4, 14, 19, 23, 25, 26, 29], but there have been disagreements about the structural changes induced in myocardial cells by a low protein diet. Some investigators reported mild or minor histological changes of myocardial cells in kwashiorkor [14, 23], whereas others have described more severe structural changes; a decrease in muscle fibre diameter, along with fewer cross-striations and vacuolization; however, one fourth of the hearts showed interstitial oedema and monocyte infiltration [2]. Both in humans and in experimental animals myocardial cells in protein malnutrition were reported to show cell atrophy, oedematous interstitial spaces, loss of cross-striation in muscle cells, small foci of necrosis and interstitial infiltration of monocytes under the light microscope [25]. Some studies, however, failed to demonstrate any structural change to the myocardial cells, except for oedema of the interstitial spaces [1]. Although a few papers dealing with the ultrastructural changes of myocardial cells in protein malnutrition have already been published [1, 23, 25, 29], very little is known about the precise ultrastructural response of the myocardial cells to a low protein diet.

The present study was carried out to reveal in detail any ultrastructural changes induced in the myocardial cells by a low protein diet and to gain an insight into factors that might be responsible for the changes. Particular attention was paid to the myofibrillar changes.

Masayo Oumi · Torao Yamamoto (✉)
Department of Anatomy and Nutrition Morphology,
Graduate School of Health and Nutrition Sciences,
Nakamura Gakuen University, 5-7-1, Befu, Jhonan-Ku,
Fukuoka 814-0198, Japan
Tel.: +81-92-851-2531, Fax: +81-92-841-7762

Materials and methods

Wistar strain male rats were used immediately after weaning. Ten rats were fed a low protein diet for 2 weeks and ten rats a control diet. Tap water was also offered ad libitum to both groups. The control diet was prepared according to the composition of AIN-93G, containing casein 20 g/100 g (20% protein), soybean oil 7.0 g/100 g, corn starch 39.7 g/100 g, dextrin 13.2 g/100 g, su-

crose 10.0 g/100 g, cellulose 5.0 g/100 g, mineral mix 3.5 g/100 g, vitamin mix 1.0 g/100 g, L-cystine 0.3 g/100 g, and choline bitartrate 0.25 g/100 g [26]. The low protein diet was identical in composition to the control diet except that it contained casein 5.0 g/100 g (5% protein) and corn starch 54.7 g/100 g in addition. Rats were weighed and their dietary consumption recorded daily. For pair feeding, 10 rats were offered a control diet in amounts restricted to the intake of the low protein diet group.

Rats were anaesthetized with ether, and part of the left ventricle was removed. Immediately after removal the material was cut into small pieces and then fixed in half-strength Karnovsky's fixative (about 1.6% paraformaldehyde and 1.7% glutaraldehyde buffered with 0.1 M phosphate, pH 7.4) for 2 h followed by postfixation in 1% osmium tetroxide in 0.1 M phosphate buffer at pH 7.4 for 1 h. After dehydration in a graded ethanol series materials were embedded in epoxy resin. Thick sections were made with glass knives on a Porter-Blum microtome, stained with toluidine blue and then examined by light microscopy. Thin sections were cut with a diamond knife on a Reichert Ultracut microtome, stained with uranyl acetate and lead, and examined in an JEOL JEM 1200EX electron microscope.

Results

The food intake efficiency rate (total body weight gain/total food intake) of a control group was 59.3±4.65%, that of a low protein group, 26.2±1.8%, and that of a pair-fed group, 50.94±1.89%. While there was a wide difference in food intake efficiency between the control group and the low protein group, no distinct difference could be seen between the control and the pair-fed groups.

As described previously [30], the myocardial cells in the left ventricle were arranged parallel to each other and sometimes branched. Most of the cytoplasm was occupied with myofibrils, often branching, running along the long axis of the cells. Large numbers of mitochondria were packed in between myofibrils. Individual myofibrils were surrounded by the sarcoplasmic reticulum and the T-tubules at the level of the Z-line. Myofibrils

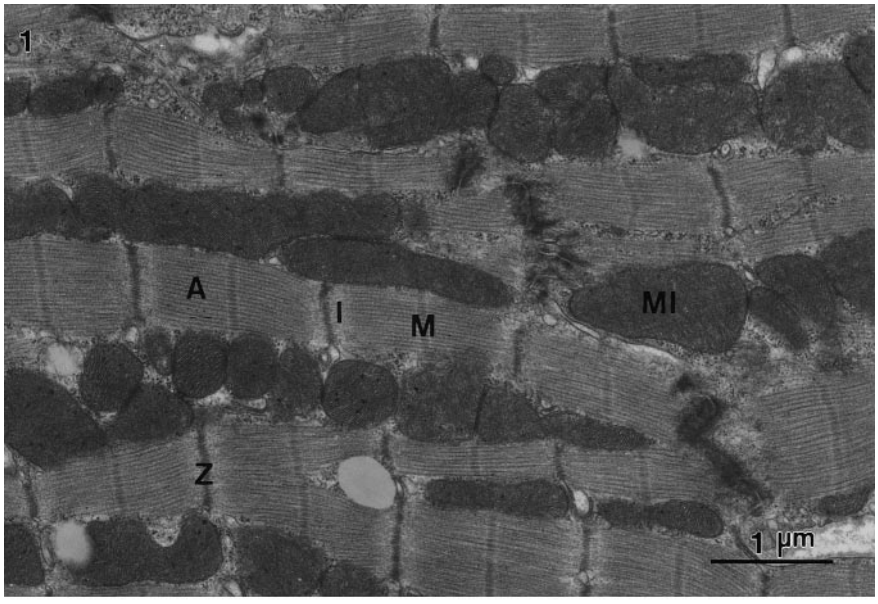

Fig. 1 An electron micrograph of the myocardial cells taken from a control rat. Myofibrils are consisted of the sarcomeres with a regular pattern of striation. In some sarcomeres the A-band (*A*), M-line (*M*), I-band (*I*) and Z-line (*Z*) are identified, but in some others the I-band are not clearly visible, presumably because of the contraction of sarcomeres. Mitochondria (*MI*) are seen tightly packed between myofibrils. ×16,000

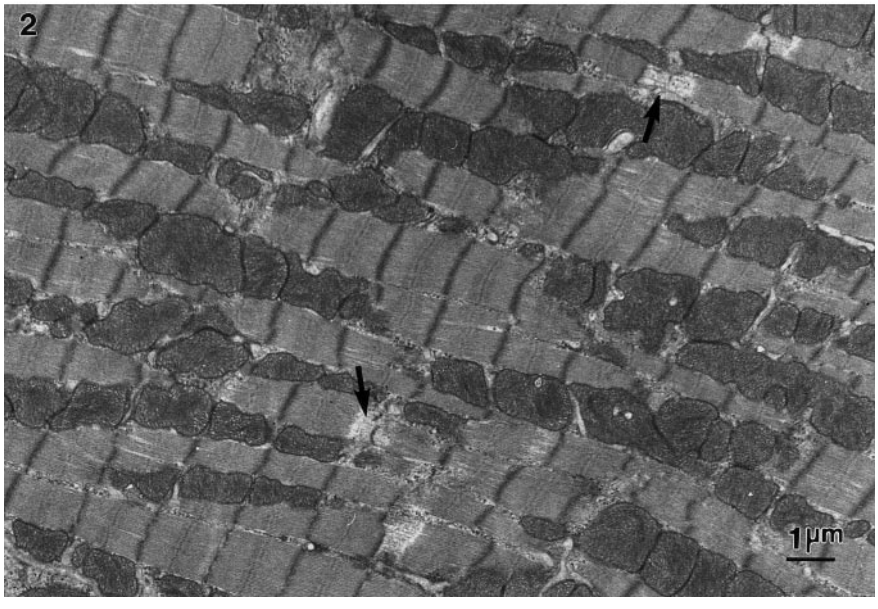

Fig. 2 An electron micrograph showing myocardial cells of the rat fed a low protein diet. The occasional fractures of myofibrils (*arrows*) are observed as the extended I-band region of the sarcomeres. ×6,000

showed a regular striation pattern, in which the A-band, M-line and I-band and the Z-line at the centre of the last, respectively, were identifiable. However, the I-band often disappeared in electron micrographs, because the sarcomere was shortened by the contraction of myofibrils during preparation of materials for electron microscopy. The intercalated discs were observed between adjoining cells, which consisted of many desmosomes in the transverse portion and of nexuses or gap junctions in the longitudinal portion (Fig. 1).

In rats fed a low protein diet, the interstitial oedema and the atrophy of muscle fibres were not so striking and cell necrosis and mononuclear cell infiltration were not detected under the light microscope. Electron microscopy, however, revealed occasional lesions of myofibrils, which were mainly confined to the I-band region of the sarcomere (Fig. 2). There were individual differences in the occurrence of these myofibrillar lesions among rats fed a low protein diet; approximately 50% of the rats examined proved to have such lesions. Even in the affected myocardial cells, myofibrils with lesions were about 10% of the total. In addition, in the myofibrils affected, the number of sarcomeres involved in the lesion varied and their individual ultrastructural changes also differed in degree and appearance (Fig. 2).

In the affected sarcomeres, the A-band was usually intact in structural features, but dislocated entirely close to the Z-line where the I-band region disappeared. However, the opposing I-band region extended to where no regular arrangement of thin filaments was clearly observed, some fine granular materials being present instead (Fig. 3). In some other sarcomeres the fractured I-band region was very markedly expanded and appeared to be empty, except for dispersing a few short filaments and granular materials closely associated with the dilated sarcoplasmic reticulum (Figs. 4, 5). In one particular sar-

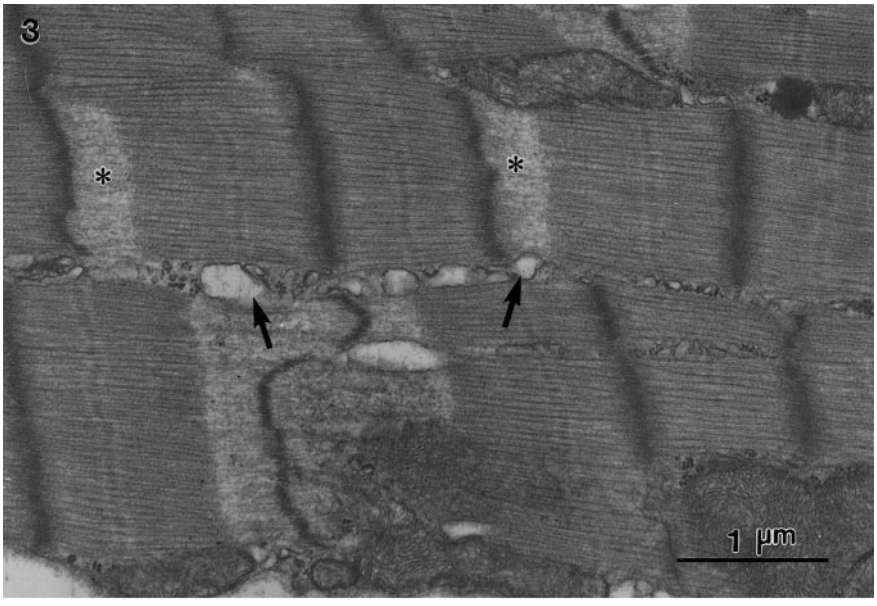

Fig. 3 The streaming of the Z-line and the extended I-band regions of two sarcomeres are seen. Two sarcomeres in the upper myofibril show the dislocation of the intact A-band close to the adjacent Z-line on one side, leaving the extended I-band region on the opposite side (*asterisks*) in close association with the dilated sarcoplasmic reticulum (*arrows*). ×20,000

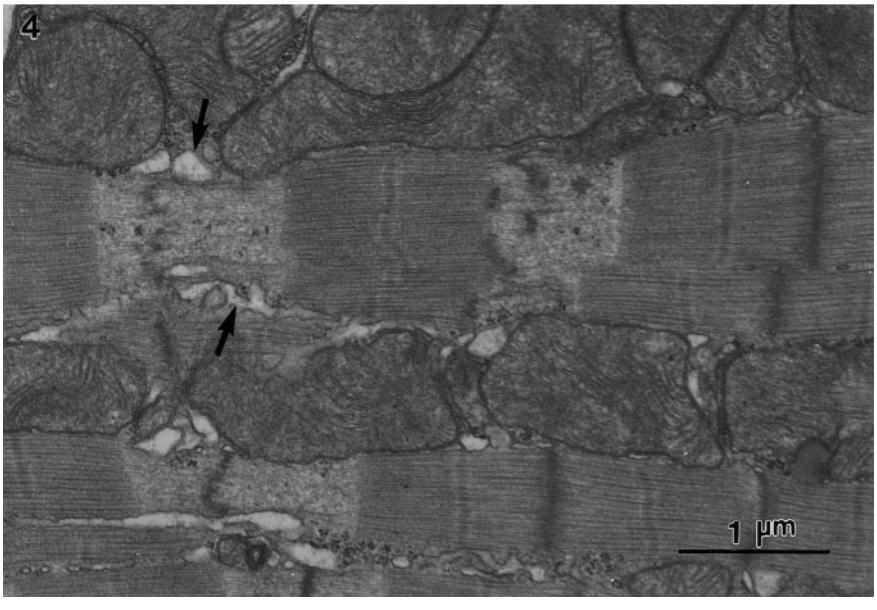

Fig. 4 The sarcomeres affected by a low protein diet show the streaming and/or disruption of the Z-line of myofibrils. These fractured I-band regions are largely expanded, lacking thin filaments, and deposited with fine granular materials. These regions are closely associated with the dilated sarcoplasmic reticulum (*arrows*). ×20,000

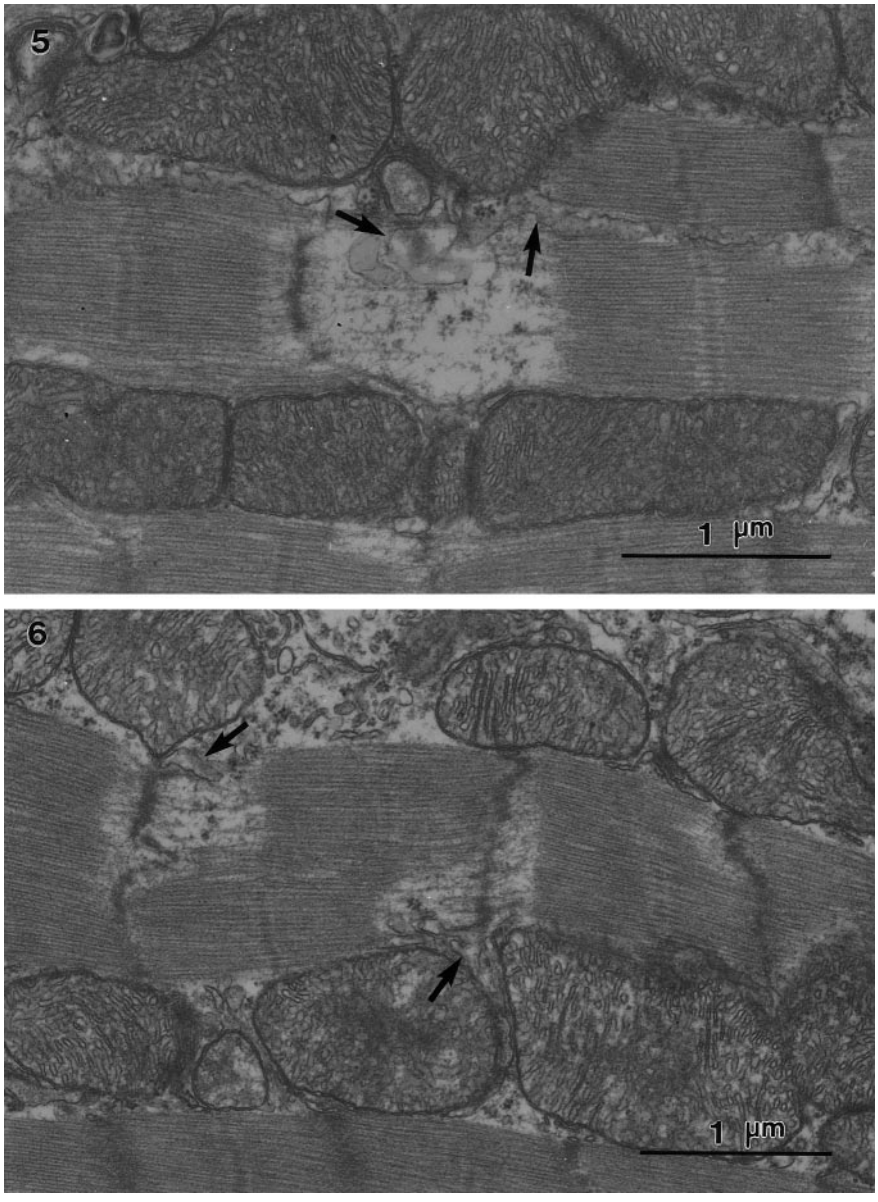

Fig. 5 This sarcomere affected by a low protein diet shows the extremely expanded I-band region from which regularly arranged thin filaments are completely absent, but fine short filaments radiate from the Z-line. The dilated sarcoplasmic reticulum is clearly seen in close association with this region (*arrows*). ×27,000

Fig. 6 This electron micrograph shows the interesting feature of myofibrillar fracture induced by a low protein diet. In one sarcomere, one half of the A-band is dislocated to one Z-line and another half to the adjacent Z-line. Both fractured I-band regions are lacking thin filaments but are closely associated with the dilated sarcoplasmic reticulum (*arrows*). ×25,000

comere, the A-band of myofibril was separated into two parts longitudinally, one half of which was closely attached to the Z-line and the other to the opposing Z-line, leaving the fractured I-band region on the each side, respectively (Fig. 6).

Most of the Z-line was generally well preserved in structural features, but in some affected myofibrils streaming of the Z-line was often observed which was accompanied by dilated sarcoplasmic reticulum (Figs. 3, 4). Some other Z-lines were ruptured to form fewer clusters, from which a few short filaments projected into the expanded empty space of the fractured I-band region (Fig. 4). In more advanced lesions of myofibrils disintegration of the sarcomere was often encountered, both actin and myosin filaments and the Z-line being disarranged and/or partially disappeared (Fig. 7). Some myofibrils showed local formation of typical contraction bands (Fig. 8). Nexus or gap junctions between adjoining cell membranes were not affected by a low protein diet.

Myocardial cells from the rats in a pair-fed group showed the same ultrastructure as did control myocardial cells. No ultrastructural changes were detected in the myocardial cells of this group.

Discussion

Although there have been disagreements on the structural changes to the myocardial cells induced by protein malnutrition [1, 2, 4, 14, 23, 27, 29, 32], the present study demonstrates that the structure of ventricular myocardial cells of rats is apparently affected by a low protein diet and also reveals that there is considerable individual difference in the degree and quantity of the ultrastructural change among the rats examined. Further, all the rats fed

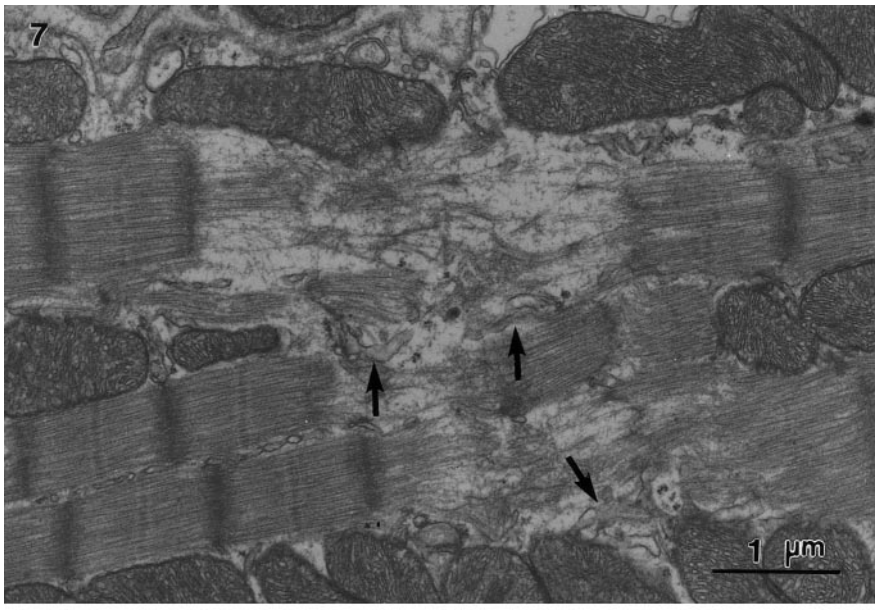

Fig. 7 The more advanced myofibrillar fractures induced by a low protein diet show the disappearance of structural feature of sacomeres and the sparsely distributed myofibrillar filaments. The dilated sarcoplasmic reticulum is present in the vicinity of these fractured regions (*arrows*). ×16,000

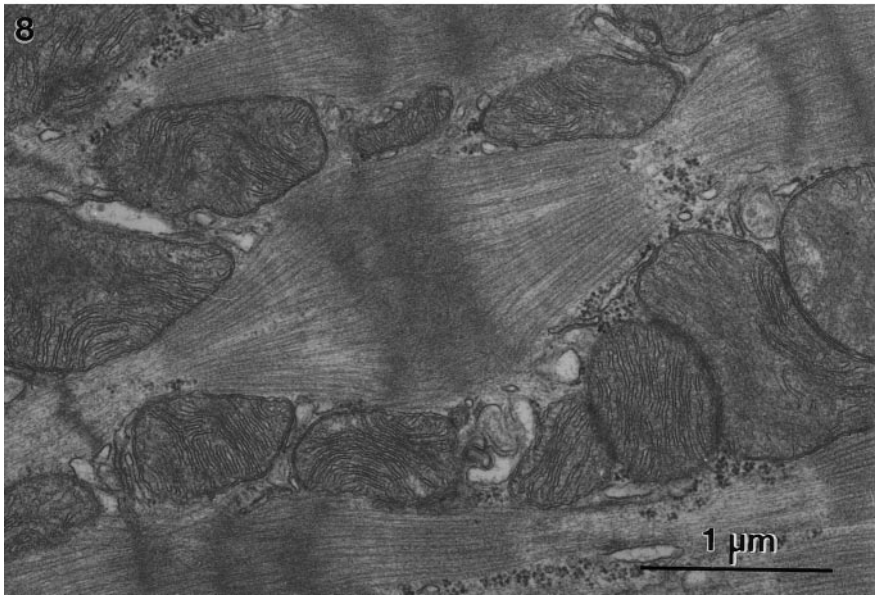

Fig. 8 The typical contraction band of myofibril induced by a low protein diet. ×25,000

a low protein diet failed to show detectable lesions on the muscle fibres, but only about 50% of them were involved in the morphological changes. The disagreements on the morphological changes seen may result, at least in part, from this individual difference. In addition, the structural changes may not be induced by a protein deficiency alone, but rather by the complex factors associated with protein metabolism and other nutrients [16, 25, 28, 39]. Therefore we cannot exclude the possibility that the differences in the composition of diet used and in experimental conditions have led to our disparate findings.

There was no significant difference in body weight gain between a control group and a pair-fed group; in addition, the latter group did not show the ultrastructural changes observed in the low protein diet group. This implies that the structural changes were induced by the low protein diet, though what mechanism was involved in the development of such changes is not known.

The ultrastructural changes in the myocardial cells of rats fed a low protein diet were characterized by occasional fracture of the myofibril confined to the I-band region of the sarcomere and by the streaming of the Z-line. This I-band fracture must be attributed to degradation of thin filaments in the I-band region and/or detachment of thin filaments from the Z-line. Recently it has been reported that myofibrils, besides both actin and myosin filaments, contain two types of giant protein filaments: titin [12, 17, 18, 23, 34, 38] and nebulin [13, 20, 37], though nebulin is absent in the sarcomere of cardiac muscle [37]. Titin filaments are now known to project from the M-line, running parallel to and in close association with thick filaments in the A-band [34] and extending into the I-band to attach to the Z-line [6, 7, 15, 24, 34]. Since titin filaments are elastic in nature [5, 7, 11, 13, 15, 17, 31, 35] and the I-band part of the titin molecules forms an elastic connector between the ends of the

thick filaments and the Z-line [7, 15, 34], the titin filaments serve to keep thick filaments centred between adjacent Z-lines [12]. Further electron microscopy has reported that in intact sarcomeres of the myocardial cells the titin filaments are laterally associated with thin filaments in the I-band, as well as with thick filaments in the A-band [6, 36].

The present observation that myofibrillar fracture at the I-band region was accompanied by dislocation of the A-band toward the adjacent Z-line, coupled with the report that the A-band is dislocated from the I-band by selective digestion of titin with trypsin [10], strongly suggests that fracture of the I-band is attributable to degradation not only of the actin filaments but also of titin filaments and, as a result, the A-band is dislocated toward the adjacent Z-line because of the elasticity of titin filaments at the opposite intact I-band region. These myofibrillar lesions may therefore lead to a low cardiac output.

In the cardiac muscle of protein malnourished rats, Rossi and Zucoloto [27] found a potential role of catecholamines in the pathogenesis of ultrastructural changes similar to those observed in the present study. We have no data pertaining to the action of catecholamines on the myocardial cells, and also it is not known at present what factors are involved in degradation of these filaments. However, since we know that such endogenous proteolytic enzymes as calpain (calcium-dependent proteinase) [22, 33] and cathepsin L (lysosomal proteinase) [19, 21] are responsible for degradation of muscle proteins, and have observed that the fractured I-band region shows few filamentous structures and/or deposition of granular materials in close association with the dilated sarcoplasmic reticulum, it is possible that the myofibrillar fracture of the I-band region is caused by digestion of actin and titin filaments with endogenous proteolytic enzymes. If this is the case, the activation of calpain must require elevation of Ca^{2+} ion and cathepsin L must need pH lower than 7 in the sarcoplasm; the conditions in myocardial cells must approximate to these values. As the turnover rate of muscle protein is markedly reduced by a low protein diet [40, 41], this reduction of turnover rate may accelerate the degradation of actin and titin filaments by modifying the susceptibility of these filaments to endogenous proteolysis.

It is suggested that free radical damage is an important factor in oedematous forms of malnutrition such as kwashiorkor [8]. Since dietary protein deficiency can result in cysteine deficiency and a resulting lack of production of glutathione, which is a potent antioxidant [8, 16], the diminished production of glutathione by a low protein diet may cause peroxidation of the cytoplasmic membranes of the myocardial cells, thus resulting in leaking of excess Ca^{2+} ion into the cytosol [8, 16, 25]. This leaked excess Ca^{2+} ion may then induce activation of calpain and formation of such a contraction band by triggering muscle contraction, as described previously [27]. As calcium appears to function in the regulation of both nonlysosomal and lysosomal proteolytic pathways in muscle [42], not only calpain but also cathepsin L are together activated by Ca^{2+} ion leaked into the cytosol.

The streaming of the Z-line is thought to be caused by degradation of cytoskeleton, perhaps the desmin connecting neighbouring myofibrils in skeletal and cardiac muscles [3]. Degradation of desmin by an enhanced action of calpain may therefore be responsible for the streaming of the Z-line.In addition, as the Ca^{2+}-induced weakening of the Z-line in postmortem muscle is thought to predominate over calpain proteolysis [9], excess leaking of Ca^{2+} ion to the cytosol may also be closely associated with the streaming of the Z-line. The present study, however, does not provide any concrete evidence to support these interpretations of the leaking of Ca^{2+} ion to the cytosol.

References

1. Abel RM, Grimes JB, Alonso D, Alonso M, Gay WA (1979) Adverse hemodynamic and ultrastructural changes in dog hearts subjected to protein-calorie malnutrition. Am Heart J 97:733–744
2. Bayo S, Kuakuvi N, Ndiae P, Quenum C (1979) Heart lesions in weaning kwashiorkor in Dakar. Report of sixty-four cases. Arch Anat Cytol Pathol 27:69–71
3. Belcastro AN, Parkhouse W, Dobson G, Gilchrist JS (1988) Influence of exercise on cardiac and skeletal muscle myofibrillar proteins. Mol Cell Biochem 83:27–36
4. Chauhan S, Nayak NC, Ramalingaswami V (1965) The heart and skeletal muscle in experimental protein malnutrition in rheusus monkeys. J Pathol Bacteriol 90:301–309
5. Funatsu T, Higuchi H, Kimura S, Ishikawa S (1990) Elastic filaments in skeletal muscle revealed by selective removal of thin filaments with plasma gelsolin. J Cell Biol 110:53–62
6. Funatsu T, Kondo E, Higuchi H, Kimura S, Ishikawa T, Yoshioka T, Maruyama K, Tsukita S (1993) Elastic filaments in situ in cardiac muscle: deep-etch replica analysis in combination with selective removal of actin and myosin filaments. J Cell Biol 120:711–724
7. Fürst DO, Osborn M, Nave R, Weber K (1988) The organization of titin filaments in the half-srcomere revealed by monoclonal antibodies in immunoelectron microscopy: a map of ten nonrepetitive epitopes starting at the Z line extends close to the M line. J Cell Biol 106:1563–1572
8. Golden MH, Ramdath D (1987) Free radicals in the pathogenesis of kwashiorkor. Proc Nutr Soc 46:53–68
9. Hattori A, Takahasi K (1982) Calcium-induced weakening of skeletal muscle Z-disks. J Biochem 92:381–390
10. Higuchi H (1992) Changes in contractile properties with selective digestion of connectin (titin) in skinned fibers of frog skeletal muscle. J Biochem 111:291–295
11. Higuchi H, Nakauchi K, Maruyama K, Fujime S (1993) Characterization of beta-connectin (titin 2) from striated muscle by dynamic light scattering. Biophys J 65:1906–1915
12. Horowits R, Podolsky RJ (1987) The positional stability of thick filaments in a activated skeletal muscle depends on sarcomere length: evidence for the role of titin filaments. J Cell Biol 105:2217–2223
13. Horowits R, Kempner ES, Bisher ME, Podolsky RJ (1986) A physiological role for titin and nebulin in skeletal muscle. Nature 323:160–164
14. Isner JM, Sours H, Paris AL, Ferrans VJ, Roberts WC (1979) Sudden, unexpected death in avid dieters using the liquid protein-modified-fast diet. Circulation 60:1401–1412
15. Itoh Y, Suzuki T, Kimura S, Ohasi K, Higuchi H, Sawada H, Shimizu T, Shibata M, Maruyama K (1988) Extensible and less extensible domains of connectin filaments in stretched

vertebrate skeletal muscle sarcomeres as detected by immunofluorescence and immunoelectron microscopy using monoclonal antibodies. J Biochem (Tokyo) 104:504–508
16. Jackson AA (1986) Blood glutathione in severe malnutrition in childhood. Trans R Soc Trop Med Hyg 80:911–913
17. Labeit S, Kolmerer B (1995) Titins: giant proteins in charge of muscle ultrastructure and elasticity. Science 270:293–296
18. Maruyama K, Matsubara S, Natori SR, Nomura Y, Kimura S, Ohashi K, Murakami F, Handa S, Eguchi G (1977) Connectin and elastic protein in muscle. Characterization and function. J Biochem 82:317–337
19. Matsukura U, Okitani A, Nishimura T, Kato H (1981) Mode of degradation of myofibrillar proteins by an endogenous protease, cathepsin L. Biochim Biophys Acta 662:41–47
20. Nutter DO, Murray TG, Heymsield SB, Uller EO (1979) The effect of chronic protein-calorie malnutrition in the rat on myocardial function and cardiac function. Circ Res 45:144–152
21. Okitani A, Matsukura U, Kato H, Fujimaki M (1980) Purification and some properties of a myofibrillar protein-degrading protease, cathepsin L, from rabbit skeletal muscle. J Biochem 87:1133–1143
22. Olson DG, Parrish FC Jr, Stromer MH (1976) Myofibril fragmentation and shear resistance of three bovine muscles during postmortem storage. J Food Sci 41:1036–1041
23. Pierobon-Bolmioli S, Betto R, Salviati G (1989) The organization of titin (connectin) and nebulin in the sarcomeres: an immunocytolocalization study. J Muscle Res Cell Motil 10:446–456
24. Pissaia O, Rossi MA, Oliveira JM (1980) The heart in protein-calorie malnutrition in rats: morphological, electrophysiological and biochemical changes. J Nutr 110:2035–2044
25. Reed DJ, Fariss MW (1984) Gluthathione depletion and susceptibility. Pharmacol Rev 36:25S–33S
26. Reeves GP, Nielsen HF, Fahey CG Jr (1993) AIN-93G purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc Writing Committee on the Reformulation of the AIN-76A Rodent Diet. J Nutr 123:1939–1951
27. Rossi MA, Zucoloto S (1982) Ultrastructural changes in nutritional cardiomyopathy of protein-calorie malnourished rats. Br J Exp Pathol 63:242–253
28. Schofield C, Ashworth A (1996) Why have mortality rates for severe malnutrition remained so high? Bull WHO 74:223–229
29. Smythe PA, Swanepoel A, Campbell JAH (1962) The heart in kwashiorkor. BMJ 13:67–73
30. Stenger RJ, Spiro D (1961) The ultrastructure of mammalian cardiac muscle. J Biophys Biocem Cytol 9:325–352
31. Stromer MH (1995) Immunocytochemistry of the muscle cell cytoskeleton. Microsc Res Tech 31:5–105
32. Svoboda D, Grady H, Higginson J (1966) The effects of chronic protein deficiency in rats. II. Biochemical and ultrastructural changes. Lab Invest 13:731–749
33. Taylor RG, Geesink GH, Thompson VF, Koohmaraie M, Goll DE (1995) Is Z-disk degradation responsible for postmortem tenderization? J Anim Sci 73:1351–1367
34. Trinick J (1994) Titin and nebulin: protein rulers in muscle? Trends Biochem Sci 19:405–409
35. Trinick J (1996) Cytoskeleton-titin as a scaffold and a spring. Curr Biol 6:258
36. Trombitas K, Granzier H (1997) Actin removal from cardiac myocytes shows that near Z line titin attaches to actin while under tension. Am J Physiol 273:C662–C670
37. Wang K, Wright J (1988) Architecture of the sarcomere matrix of skeletal matrix of skeletal muscle: immunoelectron microscopic evidence that suggests a set of parallel in extensible nebulin filaments anchorde at the Z-line. J Cell Biol 107:2199–2212
38. Wang K, McClure J, Tu A (1979) Titin: major myofibrillar components of striated muscle. Proc Natl Acad Sci USA 76:3698–3702
39. Waterlow JC (1994) Childhood malnutrition in developing nations: looking back and looking forward. Annu Rev Nutr 14:1–19
40. Waterlow JC, Stephen JML (1968) The effect of low protein diets on the turnover rates of serum, liver and muscle proteins in the rat, measured by continuous infusion of L-[^{14}C] lysine. Clin Sci (Lond) 35:287–305
41. Wykes LJ, Fiorotto M, Burrin DG, Rosario M, Frazer ME, Pond WG, Jahor F (1996) Chronic low protein intake reduces tissue protein synthesis in a pig model of protein malnutrition. J Nutr 126:1481–1488
42. Zeman RJ, Kameyama T, MatsumotoK, Bernstein P, Etlinger JD (1985) Regulation of protein degradation in muscle by calcium. J Biol Chem 260:13619–13624