Cardiac Troponin T and Creatine Kinase MB Content in Skeletal Muscle of the Uremic Rat

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Background: The assertion that creatine kinase MB (CK-MB) and the developmental isoforms of cardiac troponin T (cTnT) are expressed by skeletal muscle in some clinical settings is an extrapolation from nonuremic rodent studies. We studied the content of CK-MB and cTnT in skeletal muscle of the renal-insufficient rat.

Methods: Skeletal muscles (gastrocnemius) were collected from both five-sixths nephrectomized rats (n = 11) and sham-operated controls (n = 11). cTnT content was analyzed by Elecsys (Roche), immunoblotting, and immunohistochemistry with antibodies M7 and M11-7 (Roche). CK isoenzymes were analyzed electrophoretically.

Results: Trace concentrations of cTnT were detected in some of the skeletal muscle samples [controls (3 of 11) and uremic rats (1 of 11)] at concentrations <0.01% of that detected in heart. By contrast, positive staining appeared in both groups with M11-7 by immunoblotting and immunohistochemistry. No immunoreactivity was detected in skeletal muscle using M7 in the immunoblot format, although immunoreactivity was detected by immunohistochemistry in all samples. The median percentages of CK-MB were 6.0% and 4.1% for the skeletal muscle from control and uremic rats, respectively.

Conclusion: The detection of cTnT and CK-MB in skeletal muscle does not differ for uremic rats compared with sham-operated controls. cTnT isoforms detected by qualitative methods are not detected with the cTnT immunoassay. Observations with rodents should not necessarily be extrapolated to humans.

The use of serum creatine kinase MB (CK-MB)6 for the detection of minor myocardial damage has been superseded in recent years by cardiac troponins. However, before routine use of cardiac troponin assays, the use of CK-MB as a marker of myocardial damage was questioned in certain pathologic conditions, particularly chronic renal failure. This was because of the high frequency of increased serum CK-MB in the absence of any evidence of myocardial damage (1, 2). Increases in serum cardiac troponin T (cTnT) have also been observed without obvious evidence of cardiac damage (3–7). A hypothesis was advanced that these “false positives” were attributable to abnormal expression of cTnT and the B subunit of CK (EC 2.7.3.2) in the skeletal muscle of patients with chronic renal failure (6, 8). Central to this hypothesis were reports of increased expression of CK-MB and cTnT in skeletal muscle in various whole-animal and animal-cell line experiments. Embryonic, fetal, and in vitro growth experiments have described how myofibrillar proteins, including cTnT, have different patterns of expression in developing muscle compared with adult muscle. It is currently thought that isoforms of cTnT are transiently expressed in skeletal muscle during development. Evidence for this has been reported in various animal species (9–13). The theory that cTnT isoform expression also occurs in adult skeletal muscle is less well established. Two studies have been cited in support of this: a study of cTnT expression in normal development and in injured and denervated rat skeletal muscle (14) and a study of the C2C12 mouse muscle cell line and its response to starvation medium conditions (15).

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Received in revised form January 21, 2002, accepted March 7, 2002.

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6 Nonstandard abbreviations: CK-MB, creatine kinase MB; cTnT, cardiac troponin T; and TBS, Tris-buffered saline.
Reports have demonstrated the presence of cTnT in diseased adult human skeletal muscle at the mRNA level and at the protein level in very low, but detectable, concentrations (8,16–18). The authors of these reports have all drawn an analogy between cTnT expression and the expression of CK-MB in the skeletal muscle of the treadmill-trained rat. The B subunit of CK has been shown to be increased in expression in certain muscular dystrophies (19). However with regard to renal failure, reports that have addressed the abnormal expression of CK-MB in skeletal muscle (17,18) have discussed this in relation to a single report on the B subunit of CK in the trained rat (20). An inference in these reports is that CK-MB is abnormally expressed in skeletal muscle during training and, therefore, that cTnT is also abnormally expressed in skeletal muscle in uremia. The discussion of the abnormal expression of CK-MB has been firmly linked to the development of this theory of fetal protein reexpression in uremia. The authors of these reports have discussed this in relation to cTnT expression and, therefore, that cTnT is also abnormally expressed in skeletal muscle in uremia. The discussion of the abnormal expression of CK-MB has been firmly linked to the development of this theory of fetal protein reexpression in uremia. The discussion of the abnormal expression of CK-MB has been firmly linked to the development of this theory of fetal protein reexpression in uremia.

Although the rat has been fundamental to the development of this theory of fetal protein reexpression in uremia, analysis of these cardiac proteins in skeletal muscle has not been reported for the renal-insufficient rat. The uremic rat is more representative of human chronic renal failure than the treadmill-trained rat, the denervated and injured skeletal muscle, or the C2C12 mouse cell line. The aim of this study was to investigate the effect of uremia on the expression of cTnT and CK-MB in the rat. We qualitatively and quantitatively measured cTnT and CK-MB in heart and skeletal muscle samples taken from five-sixths nephrectomized rats (uremic rat) and sham-operated rats (controls) to detect differences in the content of cTnT and CK-MB in striated muscle in a rat model of uremia.

**Materials and Methods**

Monoclonal antibodies M7 and M11-7, used in the Roche second- and third-generation assays for cTnT, were used to probe for cTnT immunoreactivity (21) by immunoblotting and immunohistochemistry. The cTnT assay (Elecsys 1010; Roche) was used for all quantitative measurements of cTnT (Roche) in muscle extracts. Antibodies M7 and M11-7 were provided as a gift from Dr. Klaus Hallermayer of Roche (Penzberg, Germany).

**FIVE-SIXTHS NEPHRECTOMY OPERATION**

Male Wistar rats, weighing 200–220 g, were housed in individual cages and maintained at a constant temperature of 22 °C with a 12-h light-dark cycle. The animals received standard protein rat chow and tap water ad libitum. Renal impairment was produced by a two-stage five-sixths nephrectomy. Surgical procedures were performed in accordance with the method of McMahon et al. (22). Briefly, animals were anaesthetized with hypnorm and diazepam, and a midline incision made (day 0). The left kidney was exposed and decapsulated, and the renal vessels were clamped. Two-thirds of the renal cortex, including the two poles, were then removed, and pressure was applied to the surfaces before removing the clamp. After cessation of bleeding, the renal remnant was replaced in its original position, and the incision was closed. One week later (day 7), the right kidney was decapsulated and removed via a flank incision. Sham-operated control animals were prepared in a nearly identical two-stage manner, with the exception being that the kidneys were decapsulated and replaced without removing any of the cortex. Each control animal was weight-matched to a uremic partner on day 0. Pair feeding ensured that control animals were fed only the weight of food consumed by their uremic partner on the previous day. All procedures were performed in accordance with the guidelines laid down by the Home Office of Great Britain and Northern Ireland.

**SAMPLES**

Gastrocnemius muscle biopsies were taken from nephrectomized (n = 11) and control (n = 11) rats 8 weeks after surgery. In addition to skeletal muscle, left ventricle biopsies were taken from three nephrectomized and three control animals. These samples were used as positive controls for the analytical measurements. All muscle samples were initially snap-frozen and then stored at −80 °C before analysis. Crude preparations were made of the cytosolic and myofibrillar compartments of each sample. Blood samples were also collected from nephrectomized and control animals at the time of death. These samples were spun and serum stored frozen until analysis.

**PREPARATION OF MUSCLE EXTRACTS**

Muscle samples were coarsely ground in liquid nitrogen with a mortar and pestle over dry ice. Intact portions of unthawed muscle were retained for immunohistochemistry experiments. Coarsely powdered muscle was then divided into two portions: one aliquot was homogenized for Western blotting, whereas the other was homogenized for measurement of the tissue content of cTnT, CK activity, and CK isoenzymes.

Cytosolic and myofibrillar extracts of muscle samples were prepared as described previously (23). Briefly, this involved homogenization of muscles followed by three centrifugal spins at 100 000g to obtain the cytosolic fraction. The remaining pellet, containing the myofibrillar proteins, was then solubilized by incubating for 1 h with Tris buffer containing 8 mol/L urea. This fraction was then washed three times with this buffer by centrifuging at 20 000g. Urea was removed before all biochemical analyses with PD-10 Sephadex cartridges (Amersham Pharmacia Biotech).

**QUANTITATIVE MEASUREMENT OF TROPOIN T AND CK ISOENZYMES**

Total protein was determined by the Bradford method (24). All quantitative values are expressed relative to total
protein. cTnT was measured by the third-generation assay, performed on an Elecsys 1010 (Roche). All heart cytosolic and urea-free myofibrillar fractions were diluted before analysis with serum diluent supplied by the manufacturer. The use of this diluent was to reduce matrix effects. Skeletal cytosolic extracts were diluted fourfold, and skeletal myofibrillar fractions were diluted threefold. All skeletal muscle samples were diluted to ~20 mg/L protein before cTnT assay. A theoretical limit of detection was obtained by applying the manufacturer’s stated limit of detection and relating this value to the total concentration of myocyte protein (i.e., 20 mg/L) analyzed. This was set at 1 μg of cTnT per gram of protein obtained with 0.01 μg/L as the assay limit of detection (21).

Quantitative measurement of total CK activity was performed with the N-acetylcysteine activated (Roche) method on a Cobas Mira (Roche) analyzer at 37 °C. Cytosolic extracts were used to measure CK isoenzymes, which were separated and quantified by agarose gel electrophoresis by the Rapid Electrophoresis (REP) system (Helena Bioscience).

We analyzed serum samples for cTnT using the Elecsys 1010. We also confirmed uremia by analyzing serum for creatinine (Jaffe reaction) and urea (urease assay), using a Mira. Serum electrolytes, albumin, and total protein were also analyzed.

The concentrations of cTnT and CK-MB in the skeletal muscle samples from the control and the nephrectomized groups were compared by a Wilcoxon test. A Kruskal–Wallis ANOVA was used to compare the cTnT and CK-MB contents of the three muscle sample types: healthy skeletal, nephrectomized skeletal, and heart. Median serum concentrations of cTnT, creatinine, and urea, as well as electrolytes, albumin, and total protein were all compared between the two groups by a Wilcoxon–Mann–Whitney test.

**IMMUNOHISTOCHEMISTRY**

Serial 20-μm-thick sections, cut on a cryostat, were mounted on gelatin-coated slides, air dried, and then stored at −80 °C until required for immunohistochemistry. Three slides were prepared for each specimen (with a minimum of five sections per slide). Sections were cut from biopsy material taken from the gastrocnemius muscles of nephrectomized and control rats. Samples of myocardium from both nephrectomized and control animals were used as positive controls.

Sections were air dried and then fixed in 40 g/L paraformaldehyde in phosphate-buffered saline (0.1 mol/L, pH 7.4) for 10 min on ice. The sections were washed in Tris-buffered saline [(TBS); 0.05 mol/L, pH 7.6], and endogenous peroxidase was inhibited by incubation for 5 min in 30 mL/L hydrogen peroxide in methanol. After being washed in TBS, the sections were preincubated for 2 h with 4% normal horse serum in TBS. For each specimen, the sections of one slide each were incubated overnight at 4 °C with either anti-cTnT M7 IgG (1:200 by volume), anti-cTnT M11-7 IgG (1:200 by volume), or diluent alone (i.e., 40 mL/L normal horse serum). The sections were then processed at room temperature by the avidin-biotin complex method. Briefly, after being washed between each step, the sections were first incubated with biotinylated horse anti-mouse IgG (1:200, by volume, in TBS for 2 h; Vector Laboratories) followed by incubation with the avidin-biotin-peroxidase complex (ABC Elite; Vector Laboratories). Bound peroxidase was then visualized by incubation in 0.08 g/L diaminobenzidine tetrahydrochloride and 0.07 mL/L hydrogen peroxide in 0.05 mol/L Tris, pH 7.2, for 10 min. The sections were then dehydrated, cleared in xylene, and mounted in preparation for examination by light microscopy.

**GEL ELECTROPHORESIS AND IMMUNOBLOTTING OF cTnT**

Muscle samples were homogenized in phosphate buffer (200 mmol/L), pH 7.4, also containing glycerol (100 mL/L by volume), EGTA (5 mmol/L), and β-mercaptoethanol (5 mmol/L) with an Ultra Turax. Tissue homogenates were electrophoresed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Skeletal muscle (15 μg of total protein) and myocardial (2.5 μg of total protein) homogenates were loaded on 10% polyacrylamide mini gels according to the method of Laemmli (25) with the Novex Xcell II system (Invitrogen BV). Size-separated proteins were then electrophoretically blotted onto nitrocellulose membranes (Amersham Pharmacia Biotech). The membranes were then blocked with 50 g/L nonfat milk powder in phosphate-buffered saline overnight at 4 °C. The membranes were then incubated with either human anti-cTnT mouse monoclonal antibodies, M11-7, or M7. The concentration of all primary antibodies was 2 mg/L. Horseradish peroxidase conjugated to goat anti-mouse IgG (Dako Ltd) was then used at a dilution of 1:1000 for the detection of specific-binding antibody. Immunoblots were developed using ECL™ substrate (Amersham Pharmacia Biotech).

**Results**

Both creatinine and urea serum concentrations were highly significantly ($P < 0.001$ for both creatinine and urea) increased in the nephrectomized group compared with the control groups. The median (interquartile range) creatinine concentrations were 66 (59–72) μmol/L and 141 (120–188) μmol/L for control and nephrectomized rats, respectively. The corresponding values for urea were 6.2 (6.0–7.8) mmol/L and 19.8 (16.8–21.8) mmol/L for control and the nephrectomized rats, respectively. There were no significant differences in serum concentrations of bicarbonate, calcium, inorganic phosphate, potassium, and sodium between the control and nephrectomized groups. Similarly, there was no significant difference in serum protein and albumin concentrations. Serum cTnT [median (range)] was significantly higher ($P = 0.007$) in the uremic group, 0.22 (0.20–0.50) μg/L, compared with the control group, 0.1 (0.05–0.18) μg/L. There were no
correlations found among serum creatinine, urea, and cTnT concentrations in serum.

We were unable to detect cTnT in skeletal muscle at a concentration \(<0.01\%\) of that detected in heart muscle, using the Elecsys assay. None of the skeletal muscle samples demonstrated detectable concentrations of cTnT in the cytosolic fractions. A positive signal was observed in 1 of the 11 nephrectomized samples and 3 of the 11 control samples in the skeletal muscle myofibrillar fractions (Table 1). These detected values, all \(<0.004\) mg/g of protein, contrasted sharply with the heart control samples. Heart extracts gave median values (range) of 0.4 (0.2–0.7) and 6.6 (4.1–12.0) mg/g of protein for the cytosolic and myofibrillar fractions, respectively.

Representative immunoblots of heart, nephrectomized, and control gastrocnemius for the two antibodies M11-7 and M7 are shown in Fig. 1. Three distinct bands of immunoreactivity were observed with M7, and four distinct bands were seen with M11-7 in heart extracts. A major band was observed in heart extracts that migrated at a molecular mass of \(\sim 39\) kDa, as detected by both antibodies M7 and M11-7. An additional secondary band at a molecular mass of \(\sim 35\) kDa was also detected. Both M7 and M11-7 revealed much fainter bands at a molecular mass slightly higher than this secondary band at 35 kDa.

M11-7 produced a faint additional band that was not observed with M7, at a lower molecular mass (\(\sim 32\) kDa).

We found no immunoreactive bands in any of the skeletal muscle samples, using the antibody M7, within the molecular mass range of 30–40 kDa. However, M11-7 revealed immunoreactive bands corresponding to an approximate molecular mass of 35 kDa, a molecular mass lower than that of normal adult cTnT. This band was present in 8 of the 11 control-rat skeletal muscles and 9 of the 11 nephrectomized skeletal muscle samples (Fig. 1).

**Table 1. Analysis of cTnT in serum and skeletal muscle from nephrectomized (uremic) and sham-operated (control) rats with two anti-cTnT antibodies (M7 and M11-7) in qualitative techniques [Western blot (WB) and immunohistochemistry (IHC)] and quantitative techniques.**

<table>
<thead>
<tr>
<th>Rat no.</th>
<th>Serum cTnT, (\mu)g/L</th>
<th>cTnT, mg/g protein</th>
<th>WB stain</th>
<th>IHC stain</th>
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<td></td>
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<td>M11-7</td>
<td>M7</td>
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<td><strong>Control</strong></td>
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<td>1</td>
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<td>2</td>
<td>0.00</td>
<td>&lt;DL</td>
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<td>3</td>
<td>0.32</td>
<td>&lt;DL</td>
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<td>5</td>
<td>0.12</td>
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<td>6</td>
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<td>8</td>
<td>0.03</td>
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<td>9</td>
<td>0.09</td>
<td>0.001</td>
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<td>&lt;DL</td>
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<td>11</td>
<td>0.49</td>
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\(^a\) <DL, below detection limit.
Immunohistochemistry showed specific sarcomeric staining for cTnT in cardiac control tissue. We also observed positive immunostaining in all of the skeletal-muscle samples with both the M11-7 and M7 antibodies (Fig. 2). Interestingly, not all skeletal muscle fibers within a muscle fiber bundle were immunopositive for cTnT (Fig. 3).

The median (range) percentage of CK-MB in striated muscle was as follows: heart, 36.8% (24.0–48.3%); skeletal samples taken from control and uremic rats, 6.0% (1.6–11.3%) and 4.1% (1.1–10.7%), respectively. There was no significant difference in CK-MB content between the control and uremic rat samples (Wilcoxon–Mann–Whitney, P > 0.15). The distribution patterns of the CK isoforms are presented in Fig. 4.

Discussion

The main reason for performing this investigation was to address the role of rodent models in the theory of abnormal cTnT and CK-MB expression in skeletal muscle. Our findings that cTnT and CK-MB protein expression in skeletal muscle is unaffected by uremia have substantial implications for the interpretation of several published studies (6, 8, 17). These studies have been quoted as the foundation of the hypothesis that isoforms of cTnT may be expressed by the skeletal muscle of humans with chronic renal failure. It has been suggested that chronic renal-failure patients exhibit regenerating skeletal muscle and that this muscle expresses developmental isoforms of cTnT (17). In support of this theory, three studies demonstrating CK-MB in the treadmill-trained rat (20), cTnT in rat skeletal muscle (14), and expression of cTnT in the mouse skeletal muscle cell line C2C12 (15) have been cited. Using immunoblotting, Saggin et al. (14) demonstrated that cTnT was expressed in fetal rat skeletal muscle and that traces of cTnT isoforms could be detected in this tissue within the first few days after birth. They also detected (using immunohistochemistry) cTnT-like immunoreactivity in skeletal muscle in response to denervation and cold injury. McMahon et al. (15) demonstrated that C2C12 murine rhabdomyocytes transiently express cTnT when incubated in starvation medium. Although these two studies (14, 15) have contributed to charting the expression patterns of cTnT in developing muscle, we
consider our animal model to be more pertinent to human chronic renal failure.

It has been demonstrated that the CK-B subunit is overexpressed at the mRNA level in the treadmill-trained rat (20). This report (20) has been specifically cited in support of the theory of the abnormal expression of both CK-MB and cTnT in skeletal muscle associated with uremia. Several investigators have drawn an analogy between cTnT expression and the increased expression of CK-MB in the skeletal muscle of the trained rat (8, 16–18). Although the treadmill-trained rat study (20) has been used to support the theory that cTnT may be expressed abnormally in the skeletal muscle of patients with renal failure (17, 18), we consider the treadmill-trained rat to be an inappropriate paradigm of the human condition of renal failure.

We attempted to relate skeletal muscle content of cTnT to circulating concentrations. There was no correlation between frequency of increased serum cTnT and the frequency of a positive quantitative signal for cTnT in skeletal muscle. Circulating concentrations of cTnT were significantly higher in the uremic group than in the control group. In humans there is a high incidence of ischemic heart disease and cardiac death among patients with renal insufficiency (26). This high incidence of heart disease is reflected in the now well-established prognostic use of serum concentrations of cTnT in patients with renal failure (27). The serum median cTnT concentration for the rat control group was higher than that found in human controls (median value, 0.1 μg/L). Our data suggest that unlike human serum, normal rat serum does demonstrate a detectable background concentration. This slightly increased baseline for rat serum cTnT has previously been observed by Bertsch and coworkers (28, 29). There was no correlation between either serum creatinine or urea and cTnT, reflecting the findings of previously published studies in humans (3, 21).

We found positive immunohistochemical staining for cTnT, using both antibodies M7 and M11-7, and positive Western blot staining for M11-7 only. By immunohistochemistry, we demonstrated that not all muscle fibers within a muscle bundle expressed cTnT isoforms. This may explain the discrepancy between the immunohistochemical and immunoblotting results because the protein extracted from the nonexpressing muscle fibers would dilute the positive protein from the expressed fibers. Therefore, cTnT isoforms in the skeletal muscle extracts...
would be below the detection limit by immunoblotting. Furthermore, using the quantitative assay, we did not observe a positive signal in the corresponding skeletal muscle extracts that demonstrated positive qualitative staining. The quantitative assay is arranged in a “sandwich” format, in which the cTnT protein is immobilized between a solid-phase capture antibody (M7) and a labeled detection antibody (M11-7). The two antibodies bind to epitopes six amino acid residues apart from one another (21). This combination of the two antibodies leads to an assay that is extremely specific for the cardiac form of troponin T. Although individually the antibodies may demonstrate cross-reactivity to isoforms of the cTnT molecule using qualitative techniques, we have shown that a positive signal in the quantitative assay does not necessarily follow a positive Western blotting result. Our findings suggest that developmental isoforms of cTnT, which have molecular masses <39 kDa, may be detected with either of the antibodies (M7 and M11-7) individually in some skeletal muscle samples by Western blot analysis. However, a positive signal may not be produced in the same sample by the quantitative assay. This further suggests that the Roche assay is unlikely to produce false-positive results in serum from patients with regenerating skeletal muscle. Our findings are in agreement with the discussion of a previous report (17), which concluded that although a positive band of immunoreactivity may appear for skeletal muscle extracts by Western blotting, these samples would not produce a positive serum cTnT result. It would appear that isoforms of cTnT would most readily be detected in muscle by immunohistochemistry, followed by immunoblotting and then by the Elecsys quantitative assay.

Immunoreactivity was shown using the two qualitative techniques in the skeletal muscle with M11-7. We cannot make conclusions about the molecular nature of this source of immunobinding based solely on the data presented here. However, there is a possibility that the molecular forms that produced this interaction with M11-7 are alternatively spliced forms of the cTnT protein. Although the detection of alternatively spliced isoforms in adult human skeletal muscle is controversial (30, 31), the concept of multiple isoform expression during development is very well established. Multiple isoforms of cTnT have been extensively reported in the developing and failing heart (10, 32–39). These isoforms are synthesized as the result of posttranscriptional alternative RNA splicing, as demonstrated by cDNA analysis of fetal cardiac tissue in several animal species (32, 34), as well as in humans (40). In addition, evidence for this has previously been shown at the protein level in rat (41) and human (12) heart. With regard to skeletal muscle, evidence for these developmental isoforms of cTnT has been reported in rabbit (9), rat (10), and avian (11) skeletal muscle. Isoforms of cTnT have also been detected in human fetal skeletal muscle (12, 42), chicken embryos (13), and fetal rat skeletal muscle (14). Although present during development, these isoforms are down-regulated after birth. Thus the concept of developing skeletal muscle expressing proteins that are not normally associated with healthy adult muscle has firm foundations. There is therefore a mechanistic molecular basis for the possible varied expression of isoforms of cTnT in skeletal muscle. This body of evidence concerning variant isoforms of cTnT during development raised questions about the possibility of these developmental isoforms being expressed in adult skeletal muscle in pathologic conditions. Our study has addressed the issue of uremia and its effect on these cTnT isoforms in rat skeletal muscle.

The nephrectomized rats used here survived without renal replacement therapy. This particular model demonstrated increased serum urea and creatinine concentrations without severe changes in serum electrolyte balance. In this respect, the rat model was more akin to the less severe forms of human renal failure, rather than the extreme end-stage human condition requiring hemodialysis. The metabolic milieu of renal failure adversely affects many physiologic systems, including skeletal muscle metabolism (43–45). Uremic rats demonstrate increased skeletal muscle protein degradation (46–48) coupled with decreased skeletal muscle protein synthesis (48, 49), leading to a reduction in body weight compared with healthy rats (50). This weight loss was observed in our study. Skeletal muscle atrophy and fibrosis is thought to be a secondary or tertiary consequence of changes in electrolyte balance, induced via the endocrine system. Glucocorticoids (47, 51, 52), parathyroid hormone (45, 53, 54), and insulin-like growth factor 1 (55) are the main endocrine and paracrine substances shown to affect muscle physiology in uremia. Circulating parathyroid hormone is increased in chronic renal failure, whereas the effects of insulin-like growth factor 1 are attenuated via changes at the receptor level (56). The effects of parathyroid hormone have been shown to induce catabolism and inhibit production of new protein (54). The suppression of the effects of insulin-like growth factor 1 has a similar net effect on protein metabolism (55, 56). On the basis of evidence in the literature, relating hormonal influences to skeletal muscle protein turnover, we consider it unlikely that a significant amount of new protein (adult, developmental, or otherwise) would be manufactured in the rhabdomyocyte in the uremic state.

The extrapolation of the findings of any animal study with regard to serum markers of cardiac damage should always be performed with caution. The striated muscle distribution of both cTnT and CK-MB differs across animal species (57). CK-MB, in particular, is present in higher concentrations in the skeletal muscle of common laboratory animals compared with humans. It has been shown previously that the pattern of cTnT isoform expression in the human differs entirely from the rodent system (58). In fetal rat skeletal muscle, the cardiac isoform of troponin T constitute 30% of the total troponin T (58),
which is higher than the trace concentrations detected with human fetal skeletal muscle (59).

We have also demonstrated that the cTnT isoform pattern in rat heart differs from that in human heart. Previously, we found in human heart a single distinct band of immunoreactivity (corresponding to a molecular mass of ~39 kDa) by Western blotting with the antibodies M7 and M11-7 (23). The finding of multiple immunoreactive bands in rat heart indicates that the general cTnT expression in striated muscle in rats differs from that of human. Again, this brings into question the validity of extrapolating the findings in rodent studies to human studies with regard to abnormal cTnT expression. It has been shown previously that uremia does not affect the expression pattern of cTnT in rat myocardium (60). Our findings with rat hearts are in agreement with those of Cumming et al. (60). We observed no differences between the CK-MB or cTnT contents, both qualitatively and quantitatively, between the control and uremic rat heart samples. However, the sample number was too small (n = 3 for each group) to make statistical comparisons. The heart samples were included only as an experimental control for the analytical techniques because the main purpose of this study was not to investigate expression in skeletal muscle of cTnT or CK-MB in uremia.

It has been suggested that cTnT may be abnormally expressed in the skeletal muscle of patients with regenerating skeletal muscle diseases. However, with the use of a murine model of Duchenne muscular dystrophy, it has been shown previously that cTnT could not be detected by Western blotting with the M7 antibody (61). This report described similar findings for human skeletal muscle biopsies taken from patients with Duchenne muscular dystrophy. This was also supported by a report that showed cTnT protein to be undetected in the skeletal muscle of patients with Duchenne muscular dystrophy by the antibodies M7 and M11-7 in a Western blot format (18). We have also shown previously that cTnT expression in skeletal muscle is unaffected by renal failure in humans (23).

In a manner similar to the myofibrillar proteins, the B subunit of CK has been thought to have a different pattern of expression in certain conditions. The CK-MB content of Duchenne muscular dystrophy skeletal muscle has been shown to be increased (19). However, it has been demonstrated with skeletal muscle taken from patients with polymyositis that the CK-MB content was not higher than in control samples (8). In addition, the expression pattern in skeletal muscle of human athletes has been shown to exhibit increased concentrations of the CK-B subunit (62, 63). These studies are important investigations, but they are not directly relevant to uremia. We have previously shown that CK-MB is unaffected by uremia in humans. Our current study in rat supports our previous human study (23).

The use of only one specified skeletal muscle and only one sampling site was a limitation of this study. A generalization cannot necessarily be made to other skeletal muscles in relation to the proteins studied. Ideally, analysis should have been performed on examples of all three main muscle types (i.e., fast, slow, and mixed fiber skeletal muscle). This is especially important in light of the findings of Sabry and Dhoot (42), who showed variations in cTnT composition and in the pattern of isoform expression between different skeletal muscle types taken from adult and fetal rats. They showed a high degree of positive immunostaining for cTnT in the slow fiber muscles such as the soleus, as well as no positive staining with the muscles flexor digitorum sublimis, pectoris major, and tibialis anterior. However, they found that the “red area” of the gastrocnemius (mixed-fiber muscle) gave different results. The detection of a high frequency of positively stained fibers in certain skeletal muscles may potentially contribute to the overall higher basal serum cTnT compared with humans. However, we cannot confirm solely on the basis of the experiments presented here that the serum cTnT assay is affected by these isoforms.

In summary, the expression pattern of cTnT found in rat gastrocnemius differed considerably from the expression pattern in human skeletal muscle (exterior oblique) with the same experimental techniques and antibodies (23). However, we found no significant difference between the expression of cTnT or CK-MB in rat skeletal muscle (gastrocnemius) of uremic rats compared with the control group. We detected cTnT isoform expression in the skeletal muscle of both control and uremic rats. The 39-kDa adult cTnT protein was not detected in any of the skeletal muscle samples. We consider the nephrectomized rat a more appropriate model with which to address the issue of cTnT expression in adult skeletal muscle of human chronic renal failure, because this model provides more relevant information about rhabdomyocyte expression of cTnT in renal failure than does the transient expression of cTnT in a mouse cell line in vitro (15), the transient expression of cTnT in rat neonates (14), or the overexpression of CK-MB in response to training (20). Neither cTnT nor CK-MB is abnormally expressed in the skeletal muscle of the nephrectomized rat. Thus our current and previous (23) findings do not support the hypothesis that muscle weakness, which is sometimes associated with chronic renal failure (45, 64), leads to the regeneration of developmental isoforms of CK or cTnT. In the light of our findings in the rat, the theory of regeneration of cTnT isoforms and CK-MB in the skeletal muscle of humans with chronic renal failure should be reevaluated.

References

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