Quadriceps weakness in a family with nemaline myopathy: influence of knee angle

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**Abstract**

Nemaline myopathy is a congenital neuromuscular disorder, which primarily affects the thin filaments. Clinically the most important feature is muscle weakness; however, this weakness is poorly understood. The present investigation aimed to determine the torque angle relationship of the knee extensor muscles during in vivo muscle contractions in a family with a novel phenotype of nemaline myopathy. The results of this study show that quadriceps weakness occurs predominantly at higher knee flexion angles, but relatively normal strength was found at angles closer to full knee extension. When the relative torque angle relationships were considered, torque loss at smaller than optimum knee flexion angle was greater in the patients compared with the controls. In addition, the optimum angle for maximal quadriceps torque production was shifted towards smaller knee flexion angles in the patients. This suggests that a weakness specifically at higher knee flexion angles probably occurs as a result of adaptations consequently to the disease. Furthermore, it is important to assess muscle function at different joint positions to allow adequate interpretation of muscle weakness.

**Introduction**

Nemaline myopathy, a congenital neuromuscular disorder, is a disease of the skeletal muscle sarcomere, particularly of the thin filaments [1,2]. Five genes are associated with the disease all coding for thin filament proteins: α-tropomyosin (TPM3) [3], β-tropomyosin [4], nebulin [5,6], α-actin [7] and troponin T1 [8]. Histologically, nemaline myopathy is characterized by the presence of nemaline bodies (rods) in the muscle fibres [9]. The most obvious clinical feature is a mild to severe muscle weakness, predominantly of proximal limb muscles, but also of facial, respiratory or distal muscles. This muscle weakness may already be present at birth, but may also appear at older age and is usually slowly or non-progressive ([10], but see [10a]). Although this loss of strength may severely constrain patients in their daily life activities, information concerning this clinical symptom is limited. Until now, no controlled studies have been reported that have specified the degree of muscle weakness in humans with nemaline myopathy or the circumstances under which it occurs. Furthermore, the mechanisms involved in the muscle weakness are presently unclear.

In a recent study [11], it was demonstrated that transgenic mice with a mutation in the TPM3 gene exhibit altered force length characteristics compared with control mice, with reduced strength specifically at relatively short muscle lengths. On the basis of these results, it can be hypothesized that the reduced force-generating capacity observed in patients with nemaline myopathy may also be muscle-length-dependent. The purpose of the present investigation was to assess the relationship between knee extensor muscle strength and knee flexion angle during in vivo muscle contractions in a five-generation family with nemaline myopathy [12].
METHODS

Subjects
The present study included ten patients (four males; age, 44 ± 4 years) and eight healthy control subjects (three males; age, 46 ± 6 years). The patients and three of the control subjects were members of a five-generation family with a particular phenotype of autosomal-dominant nemaline myopathy characterized by muscle slowness in addition to proximal muscle weakness [12]. Linkage analysis showed a new genetic locus on chromosome 15q21-23 [13]. The patients and control subjects of this family were all genetically confirmed. None of the subjects had a previous history of neurological (other than nemaline myopathy), cardiovascular or musculoskeletal problems. All subjects provided written informed consent after they had received a careful explanation about testing procedures and involved risks. The local Medical Ethical Committee approved the study.

Experimental procedure
All subjects participated in one single experimental session where measurements of neuromuscular function of the quadriceps muscle were taken at different knee flexion angles. All subjects were asked to refrain from strenuous exercise for 48 h prior to the experiments. Before the experiments started, subjects were familiarized with the test procedures and trained to perform a maximal voluntary contraction (MVC) of approx. 3 s duration. Isometric knee extension torques at different knee flexion angles were measured with the subjects seated on a new custom-built computer-controlled lower limb dynamometer. The pelvis and upper body were fixed securely to the seat with the hip flexed at approx. 60° to minimize movements other than knee extension, and the subject’s shin was connected to the lever arm of the dynamometer. Torques (0.001 Nm resolution) were measured at the motor axis and are therefore independent of the length of the lever arm.

At each flexion angle, care was taken that the axis of the lever arm was always aligned with the axis of the knee joint (lateral femoral condyl). Subjects were asked to perform MVCs at 30°, 40°, 50°, 60° and 70° knee flexion (0° corresponds with full knee extension), which were randomly assigned to the right limb and separated by 2 min rest. During each voluntary effort subjects were encouraged loudly and visual feedback was allowed to achieve maximal performance. Torque signals were digitized (1000 Hz) and stored on disc for immediate and off-line analysis. To minimize possible variations in muscle temperature, room temperature was kept constant at approx. 22 °C during the testing procedure and subjects were in the room at least 45 min before the testing was begun.

Data analysis
Off-line analysis of torque recordings was performed with applications using custom Matlab software packages. Torque angle relationships were obtained from determining the peak torque during an MVC at each knee flexion angle. The optimal knee flexion angle for maximal torque production in each subject was estimated by interpolating the data from the obtained torque angle relationships (curve fitting). Furthermore, this optimum angle was set at 0° and normalized torques (relative to that at optimum) were calculated from the individual fitted curves at 10° intervals, between −30° and +30°.

Statistical analysis
A one-way ANOVA with repeated measures on knee flexion angle was used to test for differences between patients and control subjects on torque production. Post hoc simple contrast analysis was used to study differences between repeated measures. Student’s t tests were performed to test for differences between experimental groups for the optimal knee flexion angle. All data are presented as means ± S.E.M., unless otherwise indicated, and levels of significance were set at P < 0.05.

RESULTS
The mean torque-generating capacity during MVC, averaged over 30–70° of knee flexion, was significantly lower in patients compared with control subjects (125 ± 11 Nm compared with 173 ± 14 Nm respectively; P < 0.05). In addition, when separate knee flexion angles were considered, the differences between patients and controls were dependent on the knee flexion angle studied. The maximal torque-generating capacity of patients compared with control values was lowest at 70° (58 ± 6 %), increased with lower knee flexion angles (65 ± 6 % and 77 ± 7 % at 60° and 50° respectively) and was not significantly different at 30° and 40° (Figure 1).

The relation between torque production and knee flexion angle was clearly different for patients and controls. After curve fitting, the highest MVCs were obtained at significantly smaller flexion angles (47 ± 2°) in patients compared with controls (59 ± 5°; P < 0.05). When corrected for this optimum angle, patients (143 ± 12 Nm) were still significantly weaker compared with control subjects (204 ± 20 Nm; P < 0.05). In Figure 2, the estimated relative torques (normalized to maximal torque) are plotted against relative knee flexion angle (normalized to optimal angle, 0°). When the relative torques were considered at smaller than optimum knee angles, patients tended to show lower relative torques than controls (P = 0.066). For instance, when the relative torque was estimated for each individual at 20° smaller than optimum knee flexion angle, torque in patients was 76 ± 3 % of optimal torque, whereas in controls this
was 82 ± 2%. At 30°, torque was reduced to only 47 ± 8% of the optimum in the patients compared with 66 ± 3% in the controls. In addition, the range where active torque production was above 60% of optimum torque was broader in controls (70 ± 2°) compared with patients (59 ± 3°; P < 0.05).

DISCUSSION

To delineate whether muscle weakness observed in a family with a novel phenotype of nemaline myopathy [12] is muscle-length-dependent, the present study investigated the maximal voluntary torque of the knee extensor muscles at different knee flexion angles. The main finding of the present study was that patients with nemaline myopathy exhibit quadriceps weakness predominantly at higher knee flexion angles, which was accompanied by a shift in the optimum towards lower knee flexion angles.

Muscle weakness

The results of the present study clearly show a severe weakness of knee extensor muscles. These findings are in agreement with previous studies on nemaline myopathy that all show a greater degree of reduced muscle strength specifically of proximal limb muscles (for a review, see North et al. [10], but see [10a]). Until now, no study has clarified the exact nature of this muscle weakness. There seems, for instance, to be no direct relationship between the degree of weakness and the number of nemaline rods present in the biopsy samples [14,15]. Muscle atrophy, either as a consequence of the disease itself or due to disuse consequent upon the disease, may be suggested as a causal factor. However, although we have not objectively measured muscle volume, we could not observe clear clinical signs of muscle atrophy. In a recent study [12] it was shown that, whereas elderly nemaline patients have a reduced muscle mass with an increase in non-contractile tissue such as fat and connective tissue, fatty infiltration was absent or only slight in the younger patients. In agreement with these findings, it was shown in transgenic mice expressing a mutant TPM3 that hypertrophy of type II muscle fibres was apparent in relatively young mice, but...
absent in older mice, mimicking the late onset observed in humans with this mutation [16]. Since the subjects in our present study were also relatively young (i.e. approx. 45 years), it may be suggested that factors other than loss of muscle tissue may be responsible for the observed muscle weakness at early onset of the disease.

**Influence of knee flexion angle on muscle weakness**

To specify the degree of muscle weakness and to improve our understanding of the mechanisms involved in muscle weakness it is important to assess muscle function at its active range of torque production.

A change in the length tension characteristics of the muscle, leading to altered torque angle relationships, may at least in part underlie the quadriceps weakness observed in patients with nemaline myopathy. Indeed, the patients showed dramatic force loss around 60–70° knee flexion (i.e. force was 60–65 % of controls), a range that is normally considered to be around the optimum position for knee extensor torque generation [17,18]. In contrast, when studied at a much smaller flexion angle, the patients and control subjects showed similar maximal voluntary torques (Figure 1). Furthermore, our present results demonstrate a shift in the optimum angle towards smaller knee flexion angles in patients, explaining the relatively high torques at joint positions which would normally correspond with a relatively short muscle length and particular force loss at relatively long muscle lengths. The impaired quadriceps strength at higher knee flexion angles is in agreement with the clinical symptoms of these patients. Normal function is observed during standing and walking, but patients typically have problems during activities that require muscle contraction at relatively long muscle lengths, such as during rising from a chair or climbing stairs. When correcting for these differences in torque angle characteristics by comparing the maximal voluntary torque at estimated optimum angle, it becomes evident that the patients are still weaker than the control subjects (i.e. approx. 70 % of controls).

**Possible mechanisms for the angle-dependent muscle weakness**

The change in optimum knee angle is an interesting observation and it may be questioned whether this is directly related to the disease itself or rather results indirectly from adaptations as a consequence of the disease. One consideration is related to the disease and may be that the change in optimum knee angle is the result of impaired force generation at specific muscle lengths. Recent results on TPM3 transgenic mice [11] demonstrated enhanced force loss at shorter than optimum muscle lengths. This seems to contradict our findings that muscle weakness is most prominent at greater knee angles and hence at longer muscle lengths. However, when the data are normalized for optimum knee angle, we see a similar reduced torque at smaller knee angles in our patients (Figure 2). Furthermore, Figure 2 also shows that the range of active force production with different knee angles seemed somewhat reduced. Impaired force generation at relatively short muscle lengths or small knee flexion angles may be related to the ultrastructure of the muscle fibres in patients with nemaline myopathy, which can be severely disturbed ([10], but see [10a]). The presence of nemaline bodies (‘rods’) within sarcomeres and/or deranged aligning of actin and myosin filaments, for instance, would lead to impaired contractile performance at the level of the sarcomere.

Although no direct correlation between the degree of rod-containing fibres and force loss was found [14,15], a disturbed sarcomere structure may specifically impair torque generation at short muscle lengths where actomyosin as well as actin overlap is maximal. In addition, it was suggested that this might be due to poor activation, possibly resulting from reduced calcium sensitivity or low actin affinity specifically at short sarcomere length [11]. Apart from the presence of ‘rods’ within a sarcomere, long ‘rods’ across one or more sarcomeres may also be associated with a loss in the number of functional sarcomeres in series leading to a shorter ‘effective’ fibre length. Consequently, force production at shorter muscle lengths or smaller knee flexion angles would be reduced, optimum muscle length would shift toward lower muscle length, similar to that seen following immobilization [19], and the range of active force production with different muscle lengths would be reduced.

An alternative explanation for the observed changes in the torque angle relationship may be of a more adaptive nature. It may be possible that the changes in optimum knee angle are to compensate for the impaired contraction at relatively short muscle length. One may speculate that, if quadriceps contraction at short muscle length is impaired due to the disease, that is, if it were lower than normally expected, this would specifically affect normal daily life activities, such as standing and walking. Since these are frequently occurring activities in daily life, it seems likely to assume that the muscle would adjust its optimum to compensate for this impairment. It has been suggested that sarcomere number is adjusted so as to achieve an optimum sarcomere length when the muscle is experiencing a high level of tension [20]. Accordingly, the torque angle relationship would shift to a smaller knee flexion angle, leading to normal function during standing and walking, but impaired function during, for instance, stair climbing.

In conclusion, the present study has demonstrated muscle weakness of knee extensor muscles, in particular at greater knee angles, in a family with a novel phenotype of nemaline myopathy. This weakness is accompanied by a change in the optimum joint position for voluntary knee extensor strength. This altered muscle function in
patients with nemaline myopathy seems, to a large extent, to be related to adaptations in muscle structure and composition rather than to the disease itself. Finally, to determine the degree and prevent misinterpretation of muscle weakness it is important to investigate the full range of motion where torque production is possible. This may also apply to other neuromuscular disorders.

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