

EFFECT OF ACIDOSIS ON MAXIMAL ISOMETRIC TENSION OF UREMIC PERMEABILIZED FIBRES

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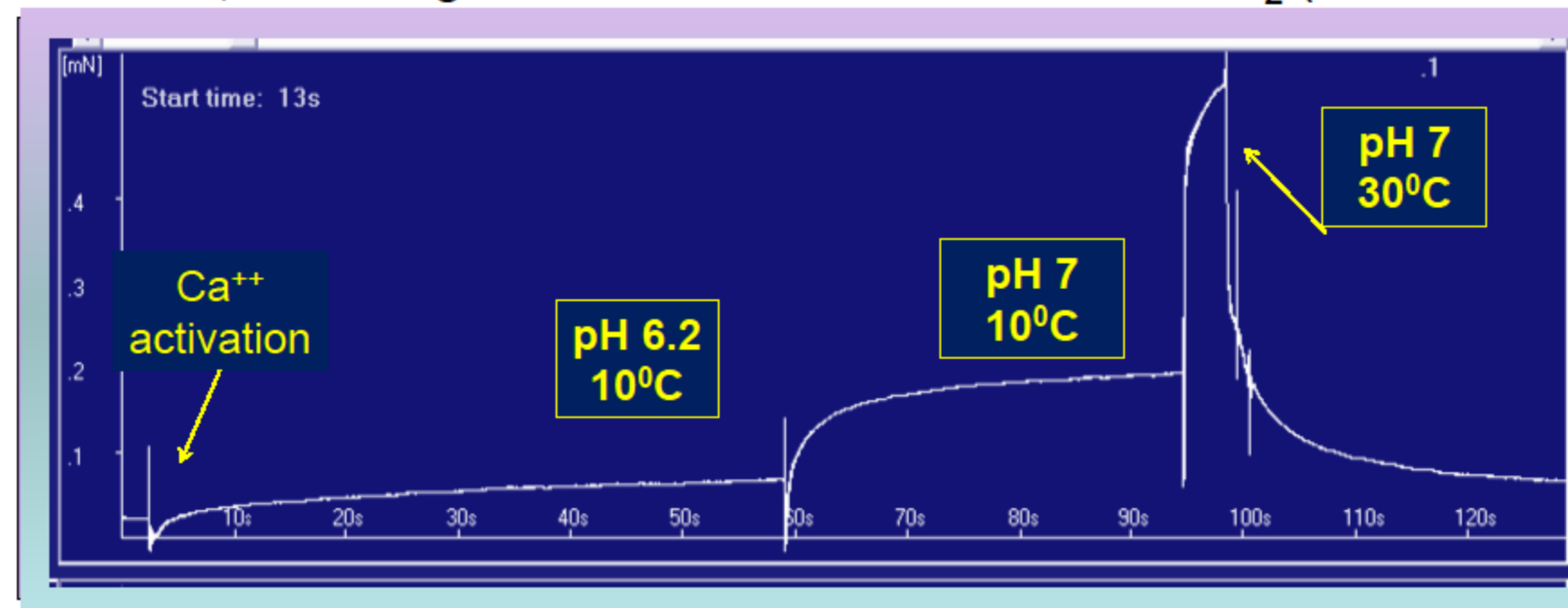
OBJECTIVES

- Chronic Renal Failure (CRF) is highly associated with muscle dysfunction, such as reduced tension generation and premature fatigue, which lead to reduced functional capacity of patients, poor quality of life and premature death (Johansen, 2007).
- Many factors may impact muscle function such as muscle's cross sectional area, substrate availability, mitochondrial function and neuropathy (Adams & Vaziri, 2006). However the causes of muscle dysfunction in this specific clinical population have not yet been understood. In order to control for confounding factors such as years in dialysis, comorbidities, pharmaceuticals, gender, nutritional status etc, we employed an animal model mimicking CRF (Gotloib et al., 1982; Bagcivan et al., 2003).
- Isometric tension is an important functional parameter (Fitts, McDonald, & Schluter, 1991) which may be assessed with the skinned fibre approach (Cooke & Bialek, 1979). The advantage of this approach is the control of selected factors (such as pH, temperature, Pi, etc.) during measurements. Thus, this approach permits to investigate the effects of uremia on tension generation capacity in different conditions.
- We aimed to evaluate the effect of acidosis on maximal isometric tension of permeabilized single psoas fibres from healthy and uremic rabbits

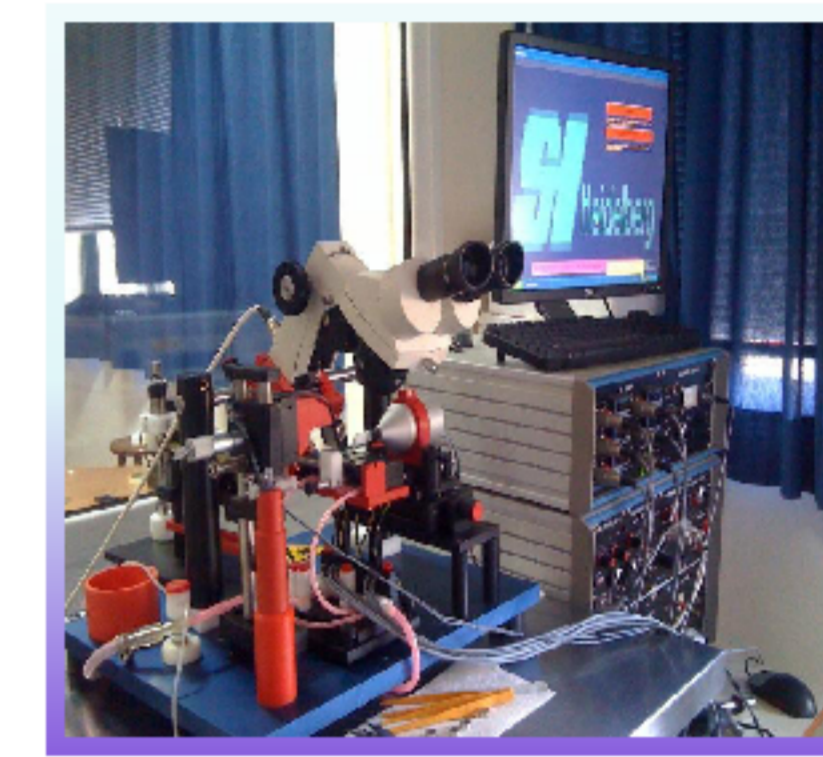
METHODS

Animal model and muscle tissue handling: We induced renal insufficiency (via partial nephrectomy) in 9 New Zealand white female rabbits. Surgery and euthanasia protocols were approved by the University of Thessaly Ethics Committee. At 3 months, psoas muscle samples harvested from sham-operated (control, CON) and uremic animals (UREM), after a 24-hour permeabilization treatment, were stored in 50% glycerol solution at -20°C until mechanical assessment.

Mechanical measurements: Isometric tension was assessed at pH 7 and pH 6.2 (acidosis) both at 10°C, and at 30°C employing the T-Jump method. Briefly, a fibre was fully activated at 10°C, pH 7 and then transferred to the 30°C, pH 7 (for 2-3 sec) and returned to relaxing buffer 10°C, pH 7. Solutions were changed and the same fibre was taken through the above steps at pH 6.2. Full activation at 10°C, pH 7 was repeated (fibres with <10% force were not considered). For some fibres assessment started with 10°C, pH 6.2 activation. This was done to avoid a possible order effect. **Solutions:** Basic rigor buffer in mM: 120 KAc, 5 MgAc₂, 1 EGTA, 50 MOPS, pH 7 or 50 MES, pH 6.2; Relaxing buffer: with addition of 5 mM ATP; Activating buffer: with addition of 1.1mM CaCl₂ (Karatzaferi et al., 2004, 2008).



Picture 1. A representative experiment of a successive activation in different conditions



Picture 2. SI Heidelberg/WPI micro dynamometer



Picture 3. T-Jump platform

Statistical analysis: An independent T-test was used to compare isometric force between the two groups in each experimental condition and the relative percentage to the force produced at 10°C, pH 7 (set as reference value). Significance level was set at p<0.05.

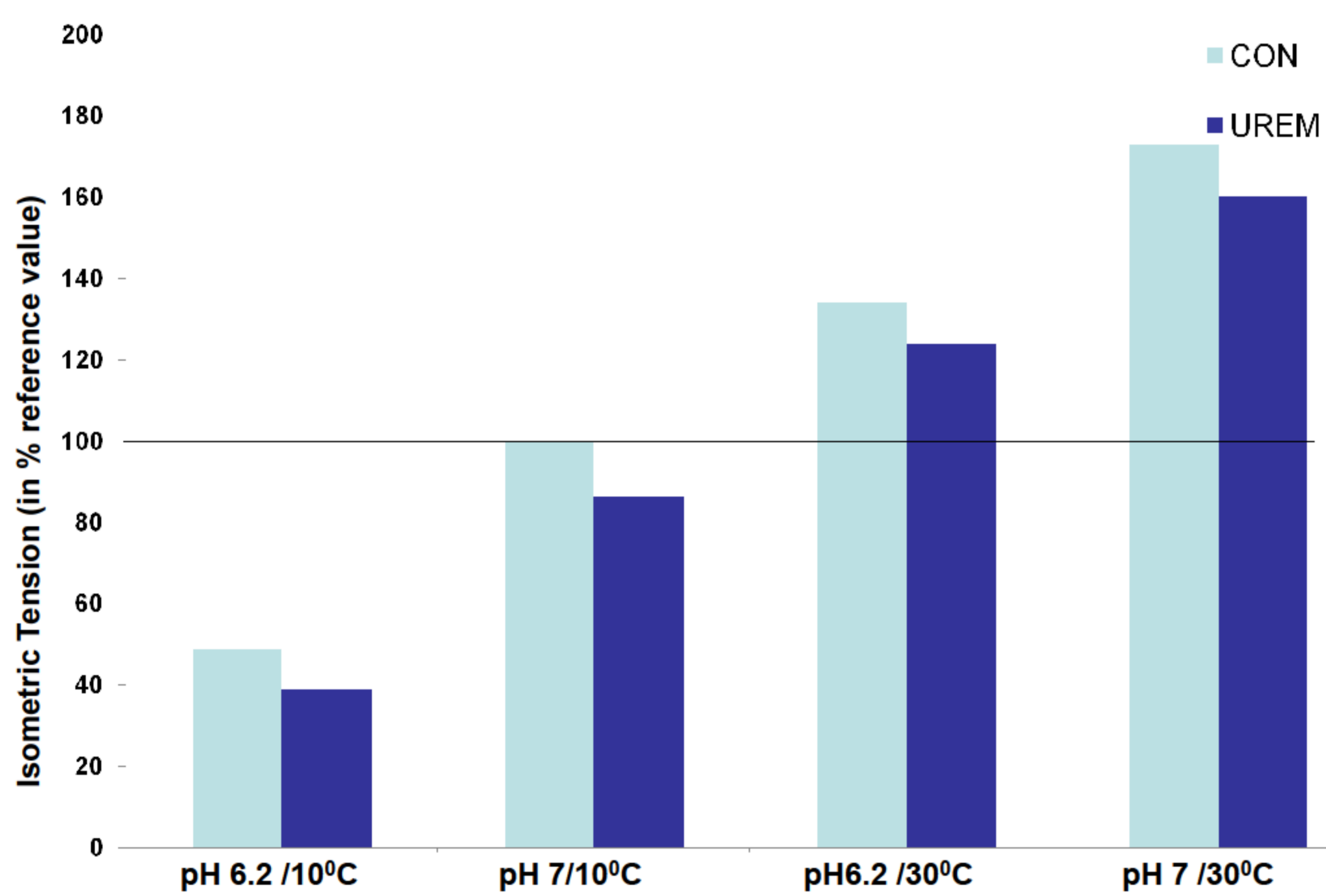


Figure 1. The average percentage of tension change with increasing temperature, expressed as percentage tension at pH 7 at 10°C (reference value) for each experimental condition for CON and UREM muscle fibers.

RESULTS

- Isometric force was lower in the UREM group fibres compared to the CON in every experimental condition.
- At the reference condition, the tension was significantly (p=0,044) higher for CON 0,074±0,032 (N=43) than 0,064±0,040 for UREM (N=78) fibres.
- At 10°C the acidosis decreased by half the relative isometric forces in both groups.
- Temperature increases (to 30°C) had a significant effect in both groups with forces increasing on average by 80% at pH 7 and 39% at pH 6.2.

Table 1. Isometric tension (μN/μm²) expressed as mean ± SD in each experimental condition (n denotes the number of fibers measured).

	pH 6.2 / 10°C	pH 7 / 10°C	pH 6.2 / 30°C	pH 7 / 30°C
CONTROL	0,039 ± 0,025 (n=43)	0,0747 ± 0,033 (n=43)	0,117 ± 0,033 (n=13)	0,177 ± 0,031 (n=12)
UREMIC	0,033 ± 0,027 (n=78)	0,0644 ± 0,04 (n=78)	0,113 ± 0,052 (n= 21)	0,151 ± 0,036 (n=20)

CONCLUSIONS

- Overall uremic muscle fibres produced lower tension than control fibres.
- In acidosis, all psoas fibres produced lower maximal isometric forces. Although tension increased with temperature, the acidosis still depressed force.
- Moreover, we noticed a large variation in the uremic force values probably reflecting some heterogeneity in this fibre population.
- In all cases, uremic fibres were characterized by decreased force compared to the control, which is in accordance to our previous preliminary work (Mitrou et al., 2013).

Acidosis seemed to affect similarly the uremic and control muscle fibres. Uremic fibres appear to have a functional deficit at resting conditions which predisposes them to worse mechanical performance in the face of muscle fatigue.

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Acknowledgments. This research has in part been co-financed by the European Union (European Social Fund - ESF) and Greek national funds through the Operational Program "Educational and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: Thales (MuscleFun Project-MIS 377260) Investing in knowledge society through the European Social Fund.

