The effects of temperature on ATPase activity and force generation in skinned muscle fibers from the Pacific blue marlin *(Makaira nigricans)*

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Summary. ATPase activity and force generation have been measured simultaneously in isolated, demembranated muscle fibers of the Pacific blue marlin *(Makaira nigricans)* between 0 and 30 °C. Tension generation is relatively independent of temperature above 15 °C and falls with a Q_{10} of < 1.5 on decreasing the temperature to 0 °C. In contrast, the Q_{10} for ATPase activity is 2.2 over the range 0–30 °C. The results are interpreted in terms of the cross bridge theory of contraction.

Key words. Marlin; muscle; mechanics; ATPase activity; temperature; skinned fibers.

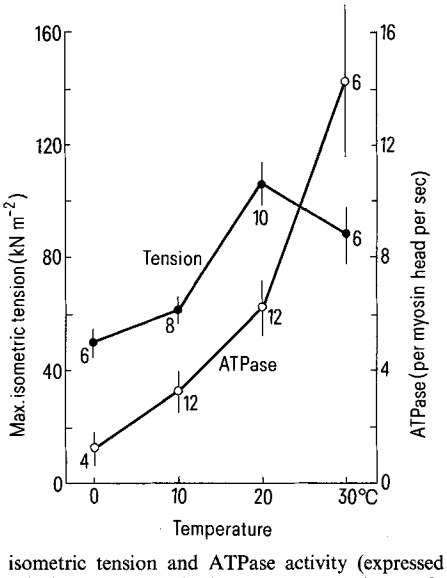
Many pelagic fishes undergo substantial vertical migration and are subject to large and relatively rapid changes in body temperature. For example, telemetric studies have shown swordfish to migrate up to 600 m in the water column in less than 2 h, experiencing a temperature change of 19 °C[2]. Swordfishes (Xiphiidae) and billfishes (Istiophoridae) have specialized 'heater' tissue associated with their extrinsic eye muscles which warm parts of the brain 10–14 °C above ambient temperature[2]. In contrast the swimming muscles operate at ambient temperature, and in the Pacific blue marlin contractile performance has been shown to be relatively independent of temperature between 15 and 25 °C.

In muscle from poikilothermic animals maximum force generation usually has a relatively low Q_{10} (1.1–1.4) over the range of temperatures normally encountered by the animal, but may decline steeply at lower temperatures[3–5]. Myofibrillar ATPase activity[6,7] and unloaded shortening speeds have different[8] and generally much higher temperature dependencies[5,6]. A limitation of previous studies on the effects of temperature on ATPase activity is that they have utilized myofibrillar suspensions which supercontract on activation, and in which the mechanical state of the myosin cross bridges is unknown and probably unphysiological. The occasion of the 26th Hawaiian International Billfish Angling Tournament enabled us to extend our studies on the muscle physiology of large pelagic gamefishes to investigate the relationship between temperature, force generation and ATPase activity in skinned muscle fibers. The myofilament lattice is preserved, and both ATPase activity and force can be measured simultaneously, and therefore under identical mechanical constraints.

Materials and methods. Pacific blue marlin *(Makaira nigricans)* (7 fish, 67–100 kg) were obtained during the 26th H.I.B.A. tournaments held at Kailua-Kona, Hawaii, during August 1984. Samples of red myotomal muscle were taken from an area near the tail of recently boated fish (15–60 min) and transferred on ice to the nearby Pacific Gamefish Foundation Laboratory. Small bundles of slow fibers up to 200 µm in diameter and 2–3 mm in length were isolated and mounted on the apparatus, a simplified version of that previously used[1]. After setting the resting sarcomere length to 2.3 µm, fiber dimensions were measured and the preparation chemically skinned with 1% brij 58 (polyoxyethylene 20 cetyl ether) in relaxing solution for 20 min at 10–20 °C. Relaxing solution had the following composition: 20 mM imidazole, pH 7.2 at 20 °C, 110 mM KCl, 3 mM $MgCl_2$, 2.5 mM ATP, and 5 mM EGTA. Maximally activating solutions were made by the addition of 4.5 mM $CaCl_2$. After skinning, the fibers were equilibrated at the experimental temperature for > 5 min, in 2 ml relaxing solution, before being transferred to a 150-µl chamber of activating solution for 3–9 min. Force was measured throughout contraction with a silicon beam strain gauge (AME, Horten, Norway). After relaxing the fiber, the activating solution was removed and frozen for later analysis in the U.K. For each bundle 2 or more activations were given at each of 2–3 temperatures between 0 and 30 °C, the temperatures being chosen in ascending or descending order. ATPase activity was determined by measuring the concentration of ADP in 100-µl samples of the pre and post contraction, activating solutions, using high performance liquid chromatography to separate and quantify the nucleotides on a reversed phase column[9]. ATPase activity is expressed in terms of ATP molecules hydrolyzed per myosin head per sec. (ATP $S_1^{-1}s^{-1}$), assuming 8% of fiber wet weight is myosin, wet weight being derived from fiber volume.

Results. The rate of ADP production at a given temperature was independent of the duration of the contraction between 3 and 9 min, showing that the results were not influenced by the time taken for ADP to diffuse out of the fiber. At 0 and 10 °C the decline in force during an experiment was typically < 1% of the maximum per min of activation. This rose to around 5% at 20–30 °C, but at the higher temperatures, contractions were usually short. There was no significant tendency towards increased or decreased ATPase during an experiment at a given temperature. The results are summarized in the figure, where maximum isometric tension and ATPase activity are plotted against experimental temperature. ATPase activity is markedly dependent on temperature, with a Q_{10} of around 2.2 over the entire range studied, but tension is much less temperature dependent, with a Q_{10} of < 1.5 below 15 °C, and around 1 between 15 and 30 °C.

Discussion. The maximum tension produced by a muscle depends not only on the absolute temperature at which it is measured, but on the relationship of this temperature to that normally experienced by the animal. Rather more data are available for fast than slow muscles. Measured at the normal body temperature of the animal, Po is usually in the range 200–400 kN m^{-2}, depending upon species, muscle and preparation. For example, in rat EDL fibers, force increases from zero at 0 °C to



Maximum isometric tension and ATPase activity (expressed as ATP molecules hydrolyzed per myosin ATPase site per se), as a function of temperature. Data expressed as mean ± SE, with the number of observations given beside each point. Data from 7 fish, 20 preparations.

340 kN m^{-2} at 22 °C, and thereafter only rises to 380 kN m^{-2} at 35 °C[10]. In contrast, fast myotomal fibers of various Antarctic fish produce 230 kN m^{-2} at −1 °C, and 260 kN m^{-2} at 10 °C[5,11]. The results obtained from the marlin are consistent with it being a relatively eurythermal animal, capable of maintaining force production over the wide range of temperatures it experiences during its diurnal vertical migrations. This appears however to be at the cost of a 2-fold decrease in 'economy' (force generated/ATP hydrolyzed) for each 10 °C rise in temperature.

Experimentally determined parameters such as Po and V_{max} can be shown to be dependent upon particular rate constants in cross bridge theory[12]. It might therefore be informative to see if the results presented are explicable on the basis of Huxley's model. Po is proportional to $f_1/(f_1+g_1)$, where f_1 and g_1 are the cross bridge attachment rate and isometric detachment rate respectively. To be consistent with the results obtained here, this relation should remain approximately constant over the physiological temperature range. The rate of isometric cross bridge turnover on the other hand is proportional to $(f_1 \cdot g_1)/(f_1+g_1)$. Isometric ATPase activity is a measure of cross bridge turnover, and is highly temperature dependent in the case of the marlin. Taking the simplest hypothesis, that the rates of attachment and detachment are both temperature dependent, and have identical Q_{10}'s, then the above equations predict to Po would be independent of temperature, and the rate of isometric cross bridge turnover would have the same Q_{10} as f_1 and g_1. The results are therefore consistent with a model in which the rates of attachment and detachment both have a Q_{10} or around 2, and the small temperature dependence observed for Po can be explained by a small difference in Q_{10} between f_1 and g_1.

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Diurnal change in stature: effects of sleep deprivation in young men and middle-aged men

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Summary. Sleep deprivation was associated with decreased stature and it blunted the normal 24-h rhythm in young and in middle-aged men. Loss in stature was regained during the first recovery night of sleep. The 24-h rhythm in height is not an endogenous circadian rhythm but depends upon the periods of recumbency over the sleep/wake cycle.
Key words. Sleep deprivation; diurnal rhythm; height, stature.

Over 200 years ago, Montbeillard found that his son's height varied according to whether he was rested or tired, and that the loss in stature by day was recovered following rest[2]. Others found the same[3–5], and that most of the decrease took place in the first hours after rising[6–8]. Diurnal variation in height may be due to variation in the water content of the nucleus pulposus[9,10], and some suggest that loss of muscle tone and associated postural changes contribute to the diurnal change in stature[11].

We undertook this study because diurnal change in stature has not been investigated using modern techniques, and because we were able to take advantage of two unrelated research projects in which volunteers were sleep-deprived (in order to study changes in blood and urine).

Methods. Study I. 12 healthy men aged 19–28 years (mean 22) were resident in the sleep laboratory for five days and nights. The first night was a baseline night with sleep, followed by 63 h of continuous sleep deprivation, which ended on the fourth evening of the study with a recovery night of sleep, followed the next evening by a second recovery night. On baseline and recovery nights, subjects were in bed from 23.00 h to 08.00 h. No subject took part in strenuous physical exercise and all were under constant surveillance.

Measurements of overall standing height, and cervical, thoracic and lumbar lengths were carried out at 08.00 h and 23.00 h of each day, beginning at 23.00 h on the first day and ending at 08.00 h on the last day. A wall-mounted Harpenden Stadiometer was used to measure stature and back length to the nearest mm. Head height was measured to the nearest mm using a craniometer. From these measurements, vertebral lengths were calculated (fig. 1). To ensure that the same vertebral point was measured at each time point, marks were made on each subject's back at C7, T12 and S2. At each measurement time, subjects were positioned standing as tall as possible[7,12], and all measurements were recorded by the same observer.

Study II. Six healthy men, aged 44–50 years (mean 47), were

Analysis of variance on measures of young men. a) Over baseline and recovery periods, showing diurnal change. b) over baseline and sleep deprivation periods showing effect of sleep deprivation treatment. c) the interaction between diurnal change and sleep deprivation

Measure	a) Diurnal change df 1,11	b) Sleep deprivation treatment df 2,22	c) Interaction df 2,22
Stature	F 88.51, $p < 0.001$	F 40.75, $p < 0.001$	F 25.04, $p < 0.001$
Cervical	NS	NS	F 8.15, $p < 0.01$
Thoracic	F 7.34, $p < 0.025$	NS	NS
Lumbar	F 15.26, $p < 0.01$	NS	F 3.64, $p < 0.05$