CHAPTER IV:

RESULTS

The effects of various lactate concentrations on whole muscle performance

At 37° C, there was no decline in force by the control muscle during the incubation period, which can be seen in the raw force data in figure 1, whereas force declined in the lactate treated muscle. Which can be seen in figure 2, where force was significantly ($p<0.05$) lower than control at 12 and 15 minutes in the lactate treated muscle,. These intervals correspond to 30 and 50 mM lactate. Figure 2, illustrates that tetanic force was reduced by 27% and 39% in the lactate treated muscle compared to the control muscle. No significant differences were noted at earlier time periods, which correspond to 10 and 20 mM lactate.

The +dP/dt depict the rate of tension development by the muscle. No significant changes in +dP/dt occurred in the control muscle. However, values were significantly reduced in the lactate treated muscles at 6, 9, 12 and 15 minutes (Figure 3). This represents lactate concentrations of 10, 20, 30 and 50 mM. The +dP/dt was lower by 21, 35, 45, 66% compared to the untreated control muscle.

The -dP/dt is representative of the rate of relaxation of the muscle. There was no significant effect changes seen in the controlmuscles or in the lactate muscles at any of the time periods (data not shown).

At 21° C there was no reduction in force production during the incubation period of the control muscles, which can be seen in figure 4. When the muscle was treated with lactate, force was significantly ($p<0.05$) lower than control at 12 and 15 minutes (figure 5), corresponding to 30 and 50 mM. There were no differences at the earlier time periods. The tetanic force declined by 8 and 9 % compared to the control muscles, which is seen in figure 5. While force was significantly reduced in the lactate treated muscles at 12 and 15 minutes at 21° C, the reduction was significantly less than at 37° C.

The +dP/dt values were also significantly reduced at 12 and 15 minutes compared to the control muscles. Figure 6 demonstrates that was a 7 and 11% reduction in the rate of tension development, when compared to the control. Again, these changes due to lactate at 21° C were significantly less than those seen at 37° C.

The -dP/dt were not altered in the control muscles or in the presence of the various lactate concentrations (data not shown).

The effects of lactate (25 mM) on Ca^{2+} release from the SR

The rate of Ca^{2+} release in the homogenate fraction of control muscles was 2.48±1.21 $\mu mol \cdot mg^{-1} \cdot min^{-1}$ (n = 11), while that of the lactate treated muscles was 1.72±0.24 $\mu mol \cdot mg^{-1} \cdot min^{-1}$ (n = 13) of free Ca^{2+}. This 31% reduction in Ca^{2+} release in the lactate group was found to be significant ($p < 0.05$). No difference was found in the amount of Ca^{2+} released between condition.

Muscle Fatigue Kinetics

To compare the reductions in force due to fatigue and that caused by 50 mM lactate, EDL muscles were repetitively stimulated using tetanic trains of pulses (0.5 ms, 100 Hz) delivered at $1 \cdot s^{-1}$ (figure 7). At both 21° C and 37° C, force rapidly declined in the first 2 minutes. This was followed by a slower decline over the next 3 minutes. As can be seen, repetitive stimulation reduced force to 7.1±0.56 and 14.8±0.06 at 37° C and 21° C, respectively.

The reductions in tetanic force due to stimulation and lactate treatment as shown in figure 8. Values were obtained at the end of the 15 minute incubation period (50 mM) and at the end of stimulation. Forces at the end of stimulation were significantly lower than those following lactate exposure.

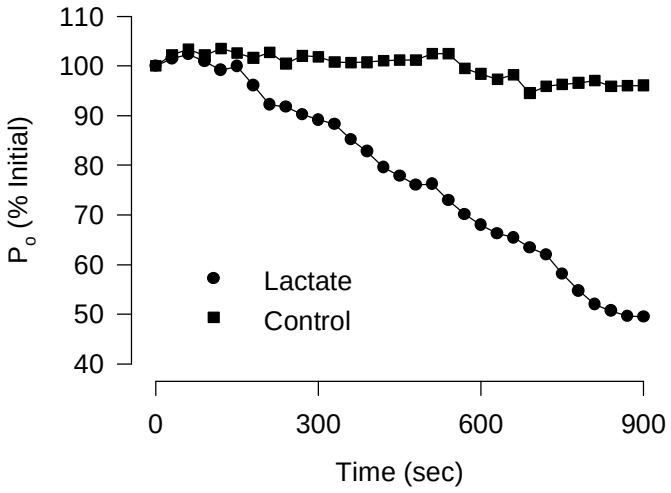

Fig. 1 Typical changes in tetanic force output by a control and lactate treated muscle at 37° C.

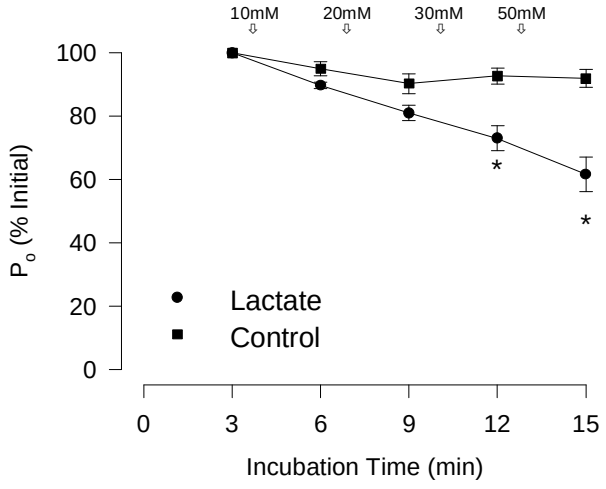

Fig. 2 The effect of increasing concentrations of lactate on tetanic force ouput at 37° C. Values are means ± SEM. (* $p < 0.05$ vs control)

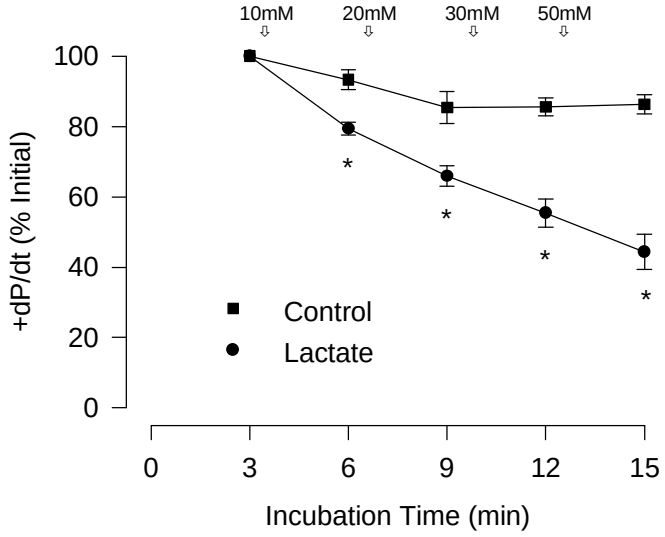

Fig. 3 The effect of increasing concentrations of lactate on the rate of tension increase at 37°C. Values are means ± SEM. (*p < 0.05 vs control)

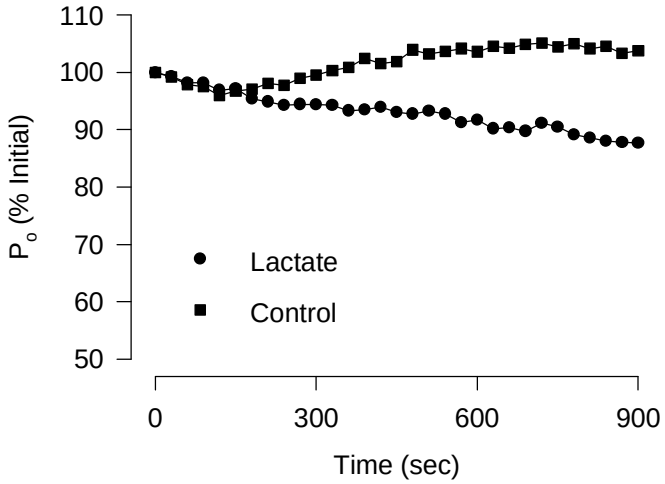

Fig. 4 Typical changes in tetanic force output by control and lactate treated muscles at 21° C.

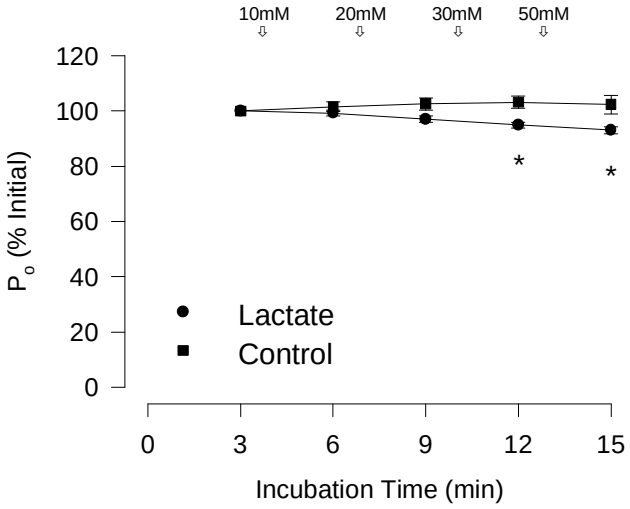

Fig. 5 The effect of increasing lactate concentrations on tetanic force ouput at 21° C. Values are means ± SEM. (* $p < 0.05$ vs control)

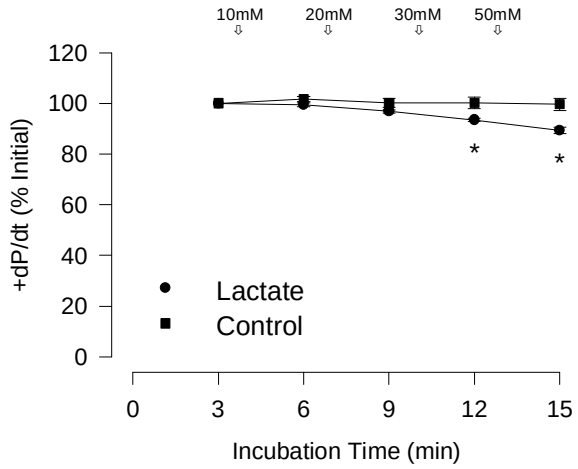

Fig. 6 The effect of increasing lactate concentrations on the rate of contraction at 21°C. Values are means ± SEM. (* $p < 0.05$ vs control)

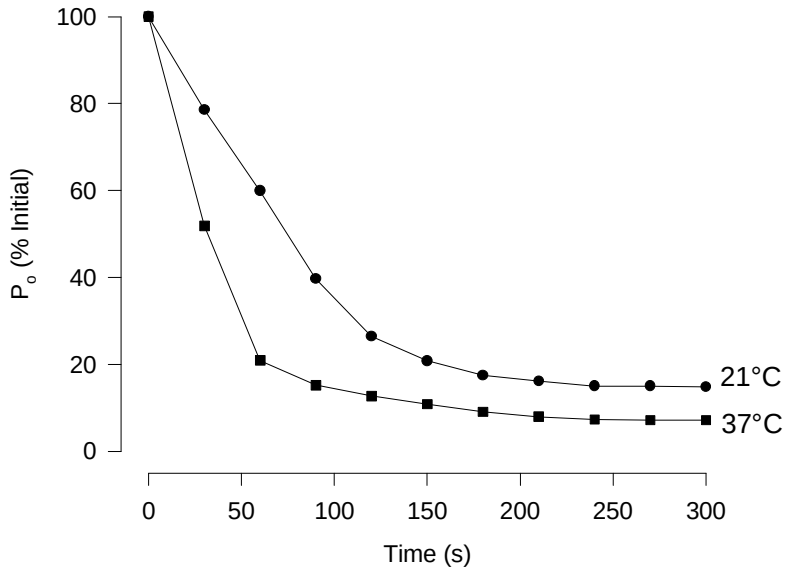

Fig. 7 Kinetics of muscle fatigue in mouse EDL. Muscles were stimulated by 100 ms trains (0.5 ms, 100 Hz) delivered at one per second.

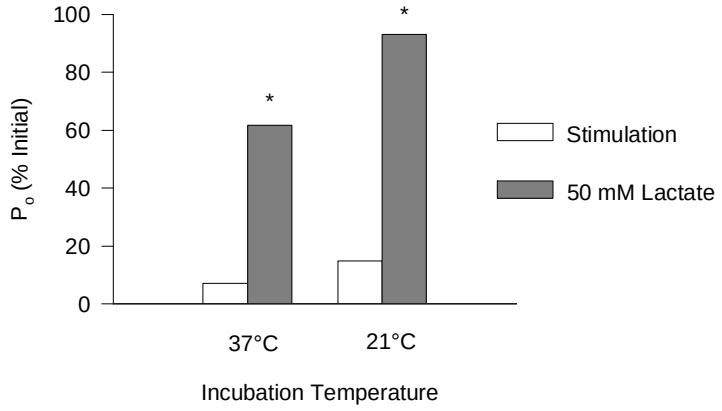

Fig. 8 Changes in tetanic force following lactate exposure (50 mM) and repetitive stimulations. ($p < 0.05$ vs lactate)

CHAPTER V:

DISCUSSION

The results indicate that lactate reduces tetanic force at temperatures near physiological (37°C) independent of pH and also confirms the reduction in tetanic force at lower temperatures (21°C). Also there was decrease in +dP/dt at both temperatures, implicating that lactate may decrease the release of Ca^{2+} from the SR. This was validated from isolated SR that lactate (25 mM) decreased the release of Ca^{2+}. This verifies that increase lactate concentrations that are seen with intense exercise are a contributing factor to muscle fatigue.

Surprisingly, few studies have examined the effects of elevated external lactate on muscle force output. Mainwood *et al.* (1986) found that an external concentration of 30 mM lactate maximum tension of frog sartorius muscle was reduced by 10-12% at a temperature of 21° C. These results are comparable to the results at 21° C found in this investigation. In contrast, Hogan *et al.* (1995) demonstrated that increased lactate concentrations had no effect on force in perfused dog muscle at physiological tempratures. However, during the repetitive stimulations it was shown that tetanic force was reduced by ~ 33% compared to 14% in the control condition. This investigation's results show that tetanic force was significantly reduced at 37° C with an external concentration of 50 mM lactate. However, in the study by Hogan *et al.* (1995) arterial lactate concentrations were 14.4 mM. It is likely that the different extracellular lactate concnetration accounts for the differences in results.

With the lactate induced reduction in force at elevated temperatures, it is possible H^+, plays some role in the decline in force production of whole muscle. When the external lactate crosses the sacrolemma into the intracellular compartment it either must carries a H^+ with it or enters undisassociated and subsequently disassociates into a lactate anion (29). This results in a reduction in intracellular pH with elevated lactate concentrations. While, several groups have shown that a decreased pH, adversely affects whole muscle function (20-22). Ranatunga (1987) and Wiseman *et al.* (1996) showed that these effects only seem to be apparent at temperatures less than 21° C. Both investigations demonstrated that force was reduced at 15° C at at pH less than 6.8. However, at the same pH they found that force increase at temperatures greater than 25° C. Since low intracellular pH has minimal effects on muscle function at 37° C, it is likely that the depressant actions of lactate found at this temperature are due to the lactate anion rather than the H^+. This contradicts the hypothesis made by Fitts (1994), that the force depressing agent is the H^+ and not lactate. Therefore, the relationship between lactate concentration and muscle fatigue may reflect possible depressant actions of lactate rather than low pH.

The mechanism for lactates effects on whole muscle performance can be gained by examining its effects on the sacroplasmic reticulum (SR) and contractile apparatus function. The present results show that Ca^{2+} release from the SR was reduced by 31% in the presence of 25 mM lactate. This confirms findings by Favero *et al.* (1995) who found that lactate concentrations of 20 mM reduced the rate of Ca^{2+} release by ~ 27% and decreased the open probability of the release channel. Favero *et al.* (1995) also showed that inhibition of the SR Ca^{2+} release channel activity by lactate was also associated with

inhibited [^{3}H]ryanodine binding by ~ 30%, changes which paralleled the reduction in the Ca^{2+} release rates (11). However, in Favero *et al.* (1995) as in the present study, measures were performed at a pH of 7.0 suggesting, reductions in SR Ca^{2+} release are the result of a direct action of lactate on the release channel. Since whole muscle force and +dP/dt was reduced in the presence of external lactate, it is possible the lactate anion may cause release channel dysfunction resulting in a reduction of the release of Ca^{2+} from the SR. With a reduction in Ca^{2+} release, full activation of the contractile apparatus would not occur and tetanic force would be reduced.

Andrews *et al*. (1996) showed that lactate also effects the functional properties of the contractile apparatus by reducing the maximal calcium-activated force (Fmax). It was found that 25 mM lactate reduced Fmax by ~ 7% of control and did not have any effect on the Ca^{2+} sensitivity (2). If the Fmax is altered significantly it would contribute to the loss of force generation by the whole muscle. As done by Favero *et al.* (1995) and in the present study the pH was maintained at 7.0. Andrews *et al.* (1996) demonstrated that the effect of lactate was concentration specific in that the greatest effect was found at 25 mM, while there was little or no effect on Fmax at 10 and 50 mM (2). Thus, lactates effects are concentration dependent with the largest depression in Fmax occurring at 25 mM. Interestingly, exposure of whole muscle to 50 mM lactate likely results in an intracellular concentration of ~ 25 mM (L.B. Gladden, personal comm.). Thus, a portion of the depression in tetanic force could be due to lactates effects on Fmax. However, it should be noted that the decline in tetanic force due to lactate exposure was greater than that observed by Andrews *et al.* (1996) on Fmax (40% vs 7%). This suggests that some additional factor is responsible for the decline in tetanic force.

It is important to emphasize that that the accumulation of lactate should not be considered as the single cause of fatigue as has been suggested by the high inverse correlations between lactate and force seen during intense exercise. When EDL muscles were fatigued at both 21° and 37° C force production declined by ~ 80% in each condition. However, when the muscle was exposed to the high external lactate concentrations tetanic force production was reduced substantially less than that seen in fatigued muscle. Thus, lactate can only account for a portion of the decline in tetanic force production during fatigue. Also, it should be pointed out that a rise in lactate is not always associated with a decline in force production and vice versa. For example, Hood *et al.* (1991) found that during the early minutes of repetitive stimulation, lactate increases and force decreases. As stimulation continues, force remains depressed while lactate levels return to normal. Taken together, the present data and the works of others suggest that the rise in intracellular lactate cannot be the sole cause of skeletal muscle fatigue. Clearly, other factors such as glycogen depletion (35), reactive oxygen species (12), or intrinsic alterations of in SR or contractile apparatus may also contribute to the decline in force production.

The major conclusion that can be drawn from this investigation is that the lactate anion reduces tetanic force production of whole muscle independent of the H^+. This seems to occur by lactate inhibiting the rate of Ca^{2+} release from the SR, thereby

preventing full activation of the contractile apparatus. However, there must be other factors involved in the fatigue process as lactate accumulation cannot fully account for the force loss during fatigue.