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# Sensitivity of ultimate meat pH to initial metabolite concentration when glycogen is not limiting.

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## ABSTRACT

The causes of variations in the ultimate pH of meat under conditions in which glycogen is in excess are considered using a model of energy metabolism in the *post-mortem* period. A key component of the model attributes the inhibition and ultimate cessation of glycolysis to the accumulation of lactate (which inhibits the regeneration of $NAD^+$ via the lactate dehydrogenase reaction) rather than acidosis. The initial concentrations (concentrations at slaughter) of creatine phosphate, ATP, lactate, and also the buffering capacity of the muscle tissue are shown to affect the ultimate pH. The ultimate pH was particularly sensitive to initial lactate concentration and muscle buffer concentration such that increases in either resulted in an increase in ultimate pH. Variations in the initial conditions of muscle metabolites are attributed primarily to pre-slaughter physical exertions, while variation in muscle buffer concentration may be genetically linked.

**Key Words:** Meat ultimate pH, initial metabolite concentration, glycogen, pre-slaughter stress

## INTRODUCTION

The management of the ultimate pH of meat is an important component of controlling the quality and consistency of meat products. In practice, ultimate pH tends to show a marked degree of variation from the desired value of 5.5 which gives optimum quality. Ultimate pH in *m. longissimus* from lambs has a skewed distribution peaking at 5.5 to 5.6, and with a range from 5.1 to 6.7 (Petersen, 1985). Similar distributions exist in beef (Smith *et al.*, 1996). In light of the impact of ultimate pH, understanding the factors that give rise to such distributions is of commercial importance.

The *post-mortem* pH changes in muscle are generally attributed primarily to glycolysis, a process in which hydrogen ions accumulate from the conversion of glycogen to lactate during the re-synthesis of ATP. Under conditions in which muscle glycogen is limiting, the extent of the *post-mortem* pH decline is restricted and the ultimate pH is elevated. Under these conditions, initial glycogen concentration will determine the ultimate pH. In conditions in which muscle glycogen is ample, typically above 50 mmol/g (Purchas and Keohane, 1997), glycolytic activity ceases before all the muscle glycogen is consumed, and hence initial glycogen no longer determines the ultimate pH. This paper examines sources of variations in the ultimate pH of meat in conditions in which glycogen is not limiting. A number of linked chemical reactions are involved in muscle energy metabolism and influence the decline and the ultimate value of the pH of meat, and the complexity of the interactions suggests that modelling will be a useful tool for understanding the process.

## 1. MODEL DEVELOPMENT

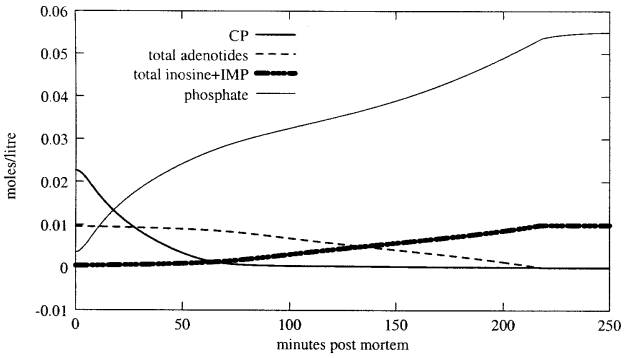
### 1.1 *Post-mortem* energy metabolism

A consistent pattern of changes in energy metabolites occurs in response to continued ATP hydrolysis in muscle during the *post-mortem* period (Figure 1). The key stages in the development of rigor mortis are:

1. Early disappearance of creatine phosphate (CP) through the creatine kinase reaction, with a stochiometric accumulation of inorganic phosphate (Pi).

2. Accelerating loss of adenosine nucleotide through the combined actions of the myokinase, deaminase and dephosphorylation reactions. These reactions result in further accumulation of Pi and depletion of adenosine nucleotides.

FIGURE 1. The change in concentration of creatine phosphate, adenine nucleotides, IMP & inosine, and phosphate in *post-mortem* muscle.




### 1.2 Buffering pH changes

1. Buffering of pH changes in muscle is derived largely from histidine residues in muscle proteins, including specific buffers such as carnosine. Buffering varies between muscle fibre types and therefore between muscles, and relates in part to carnosine concentrations.

2. The accumulation of Pi from CP and the conversion of ATP to, ultimately, inosine increases the buffering capacity of muscle as CP is depleted.

### 1.3 Rate and extent of glycolysis

1. The rate of glycolysis first increases in response to the accumulation of Pi (from CP) and accumulation of adenosine diphosphate.

2. The subsequent slowing and ultimate arrest of glycolysis is often attributed to the developing acidosis. Analysis of the glycolysis reactions indicates that it is not the accumulation of $H^+$ ions, but rather accumulating lactate that inhibits glycolysis by reducing the availability of $NAD^+$, which is in equilibrium with NADH, lactate, pyruvate and

$H^+$ through the lactate dehydrogenase (LDH) reaction (Veech *et al.*, 1979). $NAD^+$ is a necessary cofactor for the glyceraldehyde 3-phosphate dehydrogenase reaction. Thus, according to this model, the LDH reaction, and hence glycolysis, will cease when a threshold value of lactate is reached.

### 1.4 Calculation of ultimate pH

After the cessation of glycloysis, there are slight changes in pH as remaining IMP is converted to inosine, further increasing phosphate concentration. In this paper, the ultimate pH ($pH_u$) is calculated assuming all adenine nucleotides and IMP have been converted to inosine.

The rate of production of hydrogen ions, represented by *H*, can be expressed in term of the rates of change in concentrations of phosphate (*P*), creatine phosphate ($C_p$), inosine ($I_0$), IMP ($I_1$), and lactate (*L*), (Vetharaniam and Daly, in preparation):

$$\frac{dH}{dt} = \alpha\left(\gamma_P \frac{dP}{dt} + \frac{d}{dt}(C_P - I_0 - I_1 + L)\right), \quad (1)$$

where $\alpha$ represents the effect of the hydrogen buffers:

$$1/\alpha \approx \frac{k_B B}{(H + k_B)^2} + \frac{k_P P}{(H + k_P)^2}, \quad (2)$$

where *B* is the meat buffer concentration, $k_B$ and $k_p$ are the respective buffer constants of the meat buffer and phosphate ($1^-$ and $2^-$ states), and $\gamma_p$ is the fraction of free phosphate existing in the $2^-$ form in equilibrium with the $1^-$ form:

$$\gamma_P = \frac{k_P}{(H + k_P)}. \quad (3)$$

$k_B$ equals 3.2e-7 mol/litre (estimated from data in Adams *et al.* (1990)) and $k_p$ equals 2.0e-7 mol/litre Kushmerick, 1997). For the pH range being considered (7.2 to 5.4) other phosphate valences are negligible and are not considered. Furthermore, since ATP and ADP are predominantly in the magnesium-bound form, they are neglible in their buffering capacity. Define the fraction of "disassociated" muscle buffer to be:

$$\gamma_B = \frac{k_B}{(H + k_B)}, \quad (4)$$

Then, using Eqs. (2-4), and assuming temperature is constant, Eq. (1) can be integrated to give the following expression for hydrogen ion concentration, $H_f$, after rigor (Vetharaniam & Daly, in preparation):

$$H_f = 0.5k_B(B/\xi - 1) + 0.5k_P(P_f/\xi - 1) + 0.5\sqrt{(k_B + k_P - (k_B B + k_P P_f)/\xi)^2 - 4k_B k_P(1 - (B + P_f)/\xi)},$$

where

$$\xi \equiv \underbrace{\frac{k_B B}{H_i + k_B} + \frac{k_P P_i}{H_i + k_B}}_{\text{Initial dissociated Buffer conc.}} + \underbrace{C_{Pi}}_{\text{+ CP Consumed}} - \underbrace{(L_f - L_i)}_{\text{- Change in lactate conc.}} + \underbrace{I_{0f} - I_{0i} - I_{1i}}_{\text{+ Change in IMP and inosine conc.}}$$

$P_f$, $I_{0f}$, and $L_f$ are respectively the concentrations of phosphate, inosine and lactate after *rigor*, and $H_i$, $P_i$, $C_{pi}$, $I_{li}$, $I_{0i}$, and $L_1$ are the respectively the concentrations of hydrogen ions, CP, IMP and inosine at a particular reference time, $t_i$, after slaughter. When the $pH_u$ has been reached after rigor, all CP will been depleted and all ATP, ADP, AMP and IMP converted to inosine. $P_f$ and $I_{0f}$ can be calculated if concentrations of ATP, ADP, and AMP at time $t_i$,are known.

If temperature is not constant during the decline of pH, then correction terms must be added to adjust for changes in the buffer constants with temperature. For the purpose of this paper, temperature is considered constant.

$H_f$ increases ($pH_u$ decreases) with a decrease in X. Note that X, and thus $pH_u$, is dependent on the inital concentrations of CP, IMP and the adenotides, but only on the difference in lactate and inosinse concentrations. Increased initial CP or ATP increases $pH_u$, while production of lactate decreases pH. Naturally, a decrease in initial pH decreases subsequent pH, and an increase in initial pH increases subsequent pH. $L_f$ - $L_1$ is a measure of the total amount of glycolysis taking place and can be reduced by either an increase in $L_1$ or a reduction in $L_f$.

## 2. MODEL RESULTS

Table 1 shows the sensitivity of $pH_u$ to changes in substrates concentrations at slaughter for ovine *m. semitendinosus*. The intial substrate and muscle buffer concentrations for the control were obtained by fitting model paramters to $^{31}$P-NMR data (Vetharaniam, Daly & Thompson, in preparation). Initial ADP, AMP and IMP and phosphate are very small compared with ATP and are, thus, ignored.

A 10% decrease in muscle buffer concentration, *B*, results in a 0.4 drop in $pH_u$, while a 40% increase in *B* increases $pH_u$ by 0.5. By comparison, sensitivity of $pH_u$ to changes in CP is small. A 20% decrease in $C_{Pi}$ gives a decrease in $pH_u$ of 0.16 while a similar increase in $C_{Pi}$ increases $pH_u$ by 0.12. $pH_u$ showed a degree of sensitivity to pH (a 1% change in initial pH gives a 2% change in $pH_u$). Variation in initial ATP causes neglible effect, owing to the low concentration of initial ATP compared with other substrates.

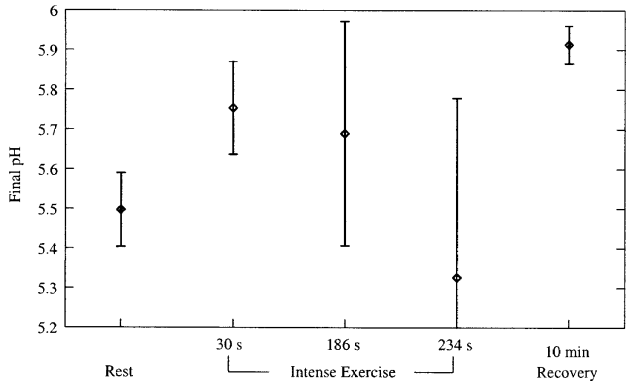
**TABLE 1:** Change in ultimate pH of meat for changes in initial substrate or buffer concentrations at slaughter, when glycogen is non-limiting.

| | Control value | Change in initial concentration of substrate | | | |
|---|---|---|---|---|---|
| | | -10% | +10% | +20% | +40% |
| Muscle buffer | 115 mM | -0.42 | 0.21 | 0.34 | 0.52 |
| | | -20% | -10% | +10% | +20% |
| Creatine phosphate | 22.7 mM | -0.16 | -0.07 | 0.07 | 0.12 |
| ATP | 9.4 mM | -0.05 | -0.02 | 0.03 | 0.05 |
| | | -1.5% | -1.0% | +1.0% | +1.5% |
| pH | 7.12 | -0.19 | -0.11 | 0.09 | 0.15 |
| | | -50% | +100% | +300% | +700% |
| Lactate | 2.8 mM | -0.04 | 0.09 | 0.22 | 0.43 |
| Final pH of control | 5.53 | Threshold lactate concentration 115 mM | | | |

Increasing the initial muscle lactate concentration reduces the total change in lactate available before the maximum lactate level (at which LDH and glycolysis is inhibited) is reached. The consequence is to reduce the total glycolytic activity that occurs *post-mortem*. Table 1 shows that small changes in the absolute value of initial lactate have significant effects on ultimate pH. Initial lactate for the control is very low (typical of well rested muscle) and an eight-fold increase in initial lactate is small with respect to potential lactate production, yet gives an increase in $pH_u$ of 0.5.

Combining small variations in several metabolites can give significant changes in $pH_u$.. Hellsten *et al.* (1999) found that 10 minutes after exercise to fatigue, CP concentration in human skeletal muscle had recovered its pre-exercise level, while ATP concentration had not, with inosine and IMP concentrations being elevated. Furthermore lactate concentration was 5.5 times as high, and muscle pH was elevated. Predictions of $pH_u$ corresponding to substrate concentrations and pH measurements made at different stages of exercise and recovery are shown in Figure 2. In the absence of phosphate and muscle buffer measurements, phosphate concentration in well-rested muscle is assumed to be 3.5 mM, and muscle buffer concentration to be 115 mM. If death occurred before exercise commenced, $pH_u$ would be 5.5, but if death occured 10 minutes into recovery from exhaustion, $pH_u$ would be 5.9. Calculated $pH_u$ scores for death occuring at different stages during exercise varied, but, because of the large experimental uncertainty for measurements during this time, these are only suggestive.

**FIGURE 2.** Ultimate pH corresponding to the substrate concentrations in human skeletal muscle at different stages of exercise till fatigue, and 10 mins into recovery.




## DISCUSSION

These results suggest that the initial metabolite concentrations in muscle can influence ultimate pH under conditions when glycogen is not limiting. By recognising that it is the accumulation of lactate, rather than $H^+$, that is responsible for the inhibition and ultimate cessation of glycolysis, the importance of the initial lactate concentration and also muscle buffering capacity in determining ultimate pH becomes apparent. Additionally, change in the threshold lactate concentration (at which glycolysis will cease) will further affect ultimate pH. Other relevant metabolites are ATP and CP, whose initial concentrations affect the amount of Pi released during their degradation and hence influence the ultimate buffering capacity of the meat.

The most likely source of variation in muscle metabolite concentration, particularly that of lactate, is physical activity immediately pre-slaughter. Vigorous physical activity produces metabolic changes similar to those seen in *post-mortem* muscle (although the rate of change will be significantly different), but some continued aerobic metabolism and the exchange of metabolites with circulating blood may generate some differences. Measurements of muscle metabolites during recovery from physical exercise clearly demonstrates how different initial conditions can be generated. In particular, the recovery of muscle pH is substantially faster than recovery of lactate concentrations to resting levels (Larsson & Hultman, 1979; Hellsten *et al.*, 1999), an effect attributed to a slower efflux of lactate compared with proton release, both during exercise and recovery (Bangsbo *et al.*, 1993).

Muscle buffering capacity varies between muscle fibre types, and species (Roos & Boron, 1981), and it is natural that muscle buffer concentration should have a statistical distribution across a population of individuals. This distribution, together with the distribution for the threshold lactate concentration, and the distributions for each of the initial metabolite concentrations, will, when combined via Eq. 5, give a calculated distribution for the ultimate pH for a given population. Knowledge of the sources of variance in ultimate pH would indicate the most efficient strategies for reducing that variance, thus offering better quality control. In particular it would allow comparison of the potential improvement through genetic selection on meat bufferring capacity versus the benefit of management strategies to reduce lactate concentrations before slaughter.

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## REFERENCES

Adams, G. R., Foley, J. M. and Meyer, R. A.1990. Muscle buffer capacity estimated from pH changes during rest-to-work transitions. *Journal of Applied Physiology. 69:* 968-972

Bangsbo, J., Johansen, L., Graham, T. E. and Saltin, B. 1993. Lactate and $H^+$ fluxes from skeletal muscles in man. *Journal of Physiology. 462*:115-133.

Hellsten, Y., Richter, E. A., Kiens, B. and Bangsbo, J. 1999. AMP deamination and purine exchange in human skeletal muscle during and after intense exercise. *Journal of Physiology 520.3*: 909-920.

Kushmerick, M. J. 1997. Multiple equilibria of cations with metabolites in muscle bioenergetics. *American Journal of Physiology. 272*: C17349-C1747.

Larsson, J. And Hultman, E. 1979. The effect of long-term arterial occlusion on energy metabolism of the human quadriceps muscle. *Scandanavian Journal of Laboratory Investigation. 39*: 257-264.

Petersen, G. V. 1985. Muscle to meat. *Publication 105*. Veterinary Continuing Education. Massey University

Purchas, R. W. and Keohane, B. E. 1997. Sources of variation in the ultimate pH of M. Longissimus from prime steers. *Proceedings of the New Zealand Society of Animal Production. 57*: 271-274.

Roos, A. and Boron, W. F.1981. Intracellular pH. *Physiological Reviews. 61*:296:434.

Smith, D. R., Wright, D. R. and Muir, P. D.1996. Variation in meat pH in

steers and associatioin with other carcass attributes: analysis of a commercial database. *Proceedings of the New Zealand Society of Animal Production. 56*: 187-192.

Veech, R. L., Lawson, J. W. R., Cornell, N. W. and Krebs, H. A. 1979. Cystosolic phosphorylation potential. *The Journal of Biological Chemistry. 245*: 6538-6547

Vetharaniam, I., Daly, C. C. and Thomson, R. A. Modelling muscle energy metabolism in resting anaerobic muscle. In preparation.

Vetharaniam, I., and Daly, C. C. Relationship between pH, temperature, and metabolite consumption in ischaemic muscle. In preparation.