Lactic Acid in Muscle and its Effects on Meat Quality

Eero J. Puolanne, A. Reeta Pösö, Marita H. Ruusunen, Katri V. Sepponen and Maria S. Kylä-Puhju)*

Motto

The only and ultimate purpose of life is to detain the entropy of solar energy. All life is to obey the rule "You shall proliferate and fill the earth!" Therefore, all reactions are optimally balanced parts of an entity, aiming to obtain the best possible ability to compete within its ecological niche. All unnecessary safety margins in capacities decrease the ability to compete. Nature has had time to optimize its processes in order to achieve this goal.

pH is the Key Factor in Relation to Meat Quality and Animal Welfare

The pH of a living muscle at rest is 7.2 to 7.4, but in severe physical and mental stress, lactic acid is produced, pH may temporarily decrease to 6.2. In living animals, the accumulation of lactic acid causes pain and distress (Gregory, 1998). Also, immediately post mortem, lactic acid will be produced as fibers attempt to keep the ATP content at the normal level (homeostasis). After several hours, post mortem meat reaches the ultimate pH, usually 5.5. This value varies within a large range and, in extreme cases, the pH may drop as low as 5.0 (Lawrie, 1998) or remain at values that are close to 7.0 (Immonen and Puolanne, 2000). This variation in pH depends mainly on muscle type and level of pre-slaughter stress, which can affect the content of available glucose. A low pre-slaughter glucose (glycogen) content in meat results in a high pH. In resting muscle, the content of glycogen is 100 mmol/kg wet weight or more, expressed as glucose. The content varies greatly depending on animal species, muscle type, feeding, and levels of stress and exercise (Immonen, 2000; Rosenvold, 2002). The buffering capacity, which also varies according to animal species and type of muscle, is ca 40 to 60 mmol/kg*pH (Kivikari, 1996). Thus it can be calculated that a decline in pH from 7.0 to 5.5 requires a formation of 60 to 80 mmol lactic acid/kg muscle tissue depending on animal species and muscle type.

It is well known that the reduction in pH by approximately 1.5 units (from the pH-level of living muscle to the ultimate value of 5.5) causes large changes to the quality/keepability of meat. Additionally, there is a strong interaction between the immediate post mortem pH-time-temperature combinations that affect meat characteristics, especially tenderness, water-holding capacity and color. However, changes in the ultimate pH that are much smaller than one unit, e.g. 0.2-0.3 pH units, resulting from 10 to 20 mmol/kg lactic acid derived from 5 to 10 mmol of glucose equivalents, are relevant, and have a marked effect on water-holding, color and tenderness. Also, rather small differences in the rate of post mortem pH decline and in temperature (Schäfer et al., 2002) have huge effects on the traits mentioned above.

In regards to animal welfare, livestock producers and animal handlers must minimize the pre-slaughter stress animals undergo. If they make no effort to do so, the result of stress may equally well be meat with an altered pH. As stated above, this change in pH may greatly reduce the quality of the meat.

The Glycogen Molecule Complex is a Masterpiece of Nature

General Model Optimizes Usefulness

The glycogen molecule contains a core protein, glycogenin (MW 37,300 Da), which supports and catalyses the synthesis of an 8 glucosyl-unit-long primer, necessary for glycogen synthase (Nelson and Cox, 2000). As glycogen synthase takes over, the chain grows and forms a branched structure. Each linear glucose chain contains 13 units, bound together with α-1,4-glycosyl bonds. At the fourth and eighth glucosyl units, each branched chain (B-chain) has a 1,6-bond which gives rise to new unbranched chains of 13 units (A-chains) (Figure 1). Consequently, after twelve branchings (13th tier) the number of chains is 1+212, or about 4,100. A glycogen molecule with the molecular weight of nine to ten million Da contains about 55,000 glu-

E.J. Puolanne
Department of Food Technology/Meat Technology
University of Helsinki
Viikki E, P.O. Box 27, FIN - 00014 Helsinki, Finland

eero.puolanne@helsinki.fi

Proceedings of the 55th Reciprocal Meat Conference (2002)

cose residues, the diameter of the molecule is close to 40 nm and the number of non-reducing ends is approximately 2,100. Each glycogen particle is covered with up to 40-50 phosphorylase dimers (or 20-25 tetramers). The structure of the glycogen molecule optimizes its usefulness: It packs the total glucose into the smallest possible volume, maximizes the number of non-reducing ends, and thus maximizes the proportion of glucose units to be removed by phosphorylase before the proximity of a branching point stops its activity (Meléndes-Hevia et al., 1993). To store 100 mM of free glucose in fibers would create such a high osmotic pressure that, energetically, it would be very expensive to maintain.

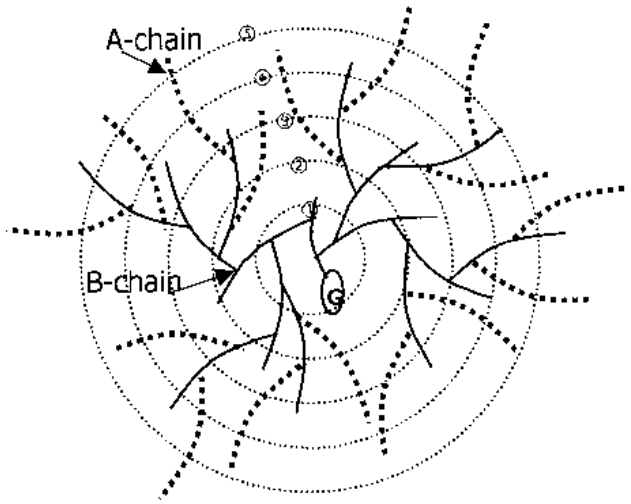

Figure 1. Scheme showing the structure of the glycogen molecule as stated in Whelan's model (Adapted from Immonen, 2000).

Proglycogen and Macroglycogen Add Complexity

The general model discussed above is, however, a simplification. The glycogen molecules exist in two forms: proglycogen (PG) and macroglycogen (MG) (Rosenvold, 2002). In resting human muscle, in which glycogen stores are full, the acid insoluble proglycogen (protein content about 10 %, MW 400,000 Da) represents approximately 60% of the total glucose (as glycogen), while in stressed muscle with less glycogen, its proportion is about 90% (Adamo and Graham, 1993). The relative amount of the acid soluble macroglycogen (0.4% protein, MW 107 Da) accounts for the rest of the total glucose content, 10-40%. In pigs, similar PG/MG ratios, approximately 68-82% to 18-32%, have been reported, the variation depending on the level of stress and feeding (Rosenvold et al., 2002).

A simple calculation based on the structure of proglycogen, assuming that each chain has 13 glucosyl residues (MW: 13*162 = 2106/chain) shows that the PG molecules have 6 (MW ca 300,000 including glycogenin) to 7 tiers (MW ca 575,000). Theoretically, this provides 64 to 128 non-reducing ends and thus sites for phosphorylase action. This also means that the number of phosphorylase units bound to each PG molecule and the number of non-reducing ends is practically the same. In comparison, the number of non-reducing ends in a MG molecule is approximately 2,100.

Phosphorylase Provides Energy for a Muscular "Explosion"

In muscle, the main sources of glucose are blood glucose and glycogen stored in muscles. In stress, hormones and/or physical stress activate phosphorylase that is able to cleave glucosyl units from a glycogen molecule at an enormous speed. The reaction cascade is explicitly described in every textbook of biochemistry and therefore, will not be presented here. This reaction sequence is under hormonal and allosterical control to allow an extremely fast production of glucose (1-P) for energy production in stress. This sequence is blocked when the influx of blood glucose meets the demand of energy, and when the enzymes of glycogen synthesis are activated (Nelson and Cox, 2000).

The glycogen phosphorylase dimers (or tetrames) are each bound at two points to A-chains of the glycogen molecules. One is active and the other is the binding site that regulates the function of the enzyme (Goldsmith et al., 1982). The phosphorylase content is very high, about 2% of the total protein in muscles (Ryman and Whelan, 1971). It cleaves glucose residues from the non-reducing end of the uppermost, unbranched A-chains, until it reaches the fourth glucosyl unit from the branching point. Glucosyl units cannot be cleaved from the end of branched B-chain that is too short (four residues). Thus, theoretically 34.6% of the units of the outermost tier are available to phosphorylase, i.e. in the case of 100 mmol/kg glucose in muscle the cleavage of about 35 mmol/kg glucose-1-P would be produced. That means formation of 70 mmol/kg lactate, if all energy was produced anaerobically (which is not the case *in vivo*). It has been shown that an increase in the number of non-reducing ends enhances, for spatial reasons, the binding of phosphorylase to the glycogen molecule and therefore, enhances the phosphorylase activity as well. The same spatial factor may limit the availablity of the glucosyl units of the lowermost tiers (Meléndes-Hevia et al., 1993).

Recently it has been shown that MG is used preferably in long-lasting aerobic stress (Asp et al., 1999), but during anaerobic stress (Graham et al., 2001) as well as post mortem (Rosenvold et al., 2002), PG is utilized more than MG. It can be speculated that the mechanism of phosphorylase activation is different in these cases. Although exact mechanisms are not known, these may involve the allosteric activation of phosphorylase b kinase by calcium ions and the allosteric activation of phosphorylase b by AMP (Nelson and Cox, 2000).

The Bifunctional Glycogen Debranching Enzyme

When phosporylase has cleaved the glucosyl units to the level of four units from the 1,6-branching point (limit dextrin), the bifunctional glycogen debranching enzyme transfers maltotriosyl groups from the 1,6-branching point (A-

chain) (transferase function) and then cleaves the remaining 1,6-glucosyl unit as free glucose (1,6-glucosidase function). This makes the further function of phosphorylase possible (Nelson and Cox, 2000). Taking both activities of the debranching enzyme into account the total activity is less than 10% of that of phosphorylase. While phosphorylase is able to cleave 92% of glucose residues, the debranching enzyme cleaves only 8% (Meléndes-Hevia et al., 1993). There is some evidence, however, that the combined activity of the debranching enzyme limits the rate of glycogen breakdown in situations where more than the uppermost layer is to be used (Nelson et al., 1969). It can also be anticipated that because of the weakened activity of phosporylase on glycogen molecules with low number of non-reducing ends, the glycogen molecule may not be fully utilized. Experimental results show that at least 10 to 20 mmol/kg glycogen is always left in muscles, even post mortem and, in well-fed and non-stressed animals the residual glycogen may be much higher, up to 80 mmol/kg, even when the ultimate pH is 5.5 (Immonen and Puolanne, 2000).

The Formation of Lactic Acid: Response to the Sudden Need for Energy

In the muscle fibers of a living animal, lactic acid is produced almost constantly. The acid formed is either converted back to pyruvic acid to be used oxidatively via the tricarboxylate acid cycle, or by lack of oxygen and/or mitochondria, moved out of the fiber. When glucose is broken down into two molecules of pyruvate, two to three molecules of ATP are generated. In addition, two molecules of NAD+ are reduced to NADH, which must be reoxidized in order to maintain the rate of glycolysis. When oxygen is available, electrons from NADH are transferred to molecular oxygen via the mitochondrial electron transport chain. However, when oxygen is lacking, oxidation of NADH is coupled with the reduction of pyruvate to lactate, i.e. lactate formation is a prerequisite for anaerobic energy production (Nelson and Cox, 2000).

Back and Forth: Lactate Dehydrogenases

Pyruvate is the junction point in carbohydrate metabolism. When oxygen is available and the density of mitochondria is not a limiting factor, the citric acid cycle and oxidative phosphorylation are the preferred pathways of oxidation and energy production. When the demand for ATP exceeds the capacity of aerobic metabolism, ATP will be produced anerobically, which, as previously explained, involves the reduction of pyruvate to lactate. The reaction is catalyzed by lactate dehydrogenases in cell cytosol (Nelson and Cox, 2000). In muscles, as in other tissues, lactate dehydrogenases occur as five tetrameric isoenzymes (LDH-1 ... LDH-5). The four units in each LDH molecule are composed of muscle-type (M) or heart-type (H) chains. The extreme combinations are H4 (LDH-1) and M4 (LDH-5) (Dawson et al., 1964; Fieldhouser and Masters, 1966). LDH-5 has a high Km for pyruvate, thus favoring the formation of lactic acid when the rate of glycolysis is high. Furthermore it is not inhibited by an excess of pyruvate as is LDH-1. The activity of heart-type LDHs, isoenzymes that contain a high proportion of H-chains, is higher in the heart than in muscles. With the M-type the situation is the opposite. Although the M-type is dominating in both types of muscles, the dark muscles contain more H-type activity when compared to light muscles. Porcine muscles have an exceptionally high LDH activity (Hamm and El-Badawi, 1991; Suuronen, 1995). The acute stress, coupled with a low capillary and mitochondrial density, will favor the formation of lactic acid, which as it is a utilizable source of energy, is later converted back to pyruvate. This takes place more in the heart muscle and dark (oxidative) muscles than in light (glycolytic) muscles.

Monocarboxylate Transporters: A New Finding in Muscle Physiology

Only a minor fraction of the lactic acid formed remains as undissociated acid. The near neutral pH in cells, together with the relatively low pK_a value of lactic acid, 3.86, favor its dissociation into a lactate anion and a proton. In muscle fibers the key enzyme of glycolysis, phosphofructokinase, and thus energy production from glucose, is inhibited by a high concentration of protons (Hyyppä and Pösö, review 1998). Buffering substances, such as bicarbonate, phosphate, protein, anserine, and carnosine tend to prevent the drop of pH and maintain energy production. Another alternative for the maintenance of pH is to transport lactic acid and/or protons out of the cell. Only lactic acid diffuses through the lipid bilayers of membranes, whereas both protons and lactate anions need the help of carrier proteins (Juel, review 1997).

Several carrier systems have been characterized to operate in muscle tissue (Juel, review 1997; Juel and Halestrap, 1999; Pösö, review 2002). Part of the protons are transported by the Na^+/H^+ exchange protein which simultaneously transports one sodium ion into the cell and one positively charged proton out of the cell. This transport system, however, disturbs the sodium/potassium balance across the membrane and increases the amount of energy needed to maintain the balanced concentrations of sodium and potassium. It is generally believed that in muscle tissue, the role of the Na^+/H^+ exchanger is only minor (Juel, review 1998). Lactate anions may be transported out of the cell by the anion exchange protein (AE), which exchanges a lactate anion for a chloride or bicarbonate anion. This carrier does not, however, influence the concentration of protons in the cell. In muscle, the major portion of carrier-mediated lactate transport occurs via the function of monocarboxylate transporters (MCT), which transport a lactate anion and a proton together according to the direction of the lactate gradient (Juel, review 1997). There are at least 9 isoforms of MCTs, which are numbered according to their discovery (Halestrap and Price, review 1999). These isoforms appear to be quite

conserved, and they are expressed in a tissue and species specific manner (Halestrap and Price, review 1999; Bonen, review 2000). Most thoroughly characterized are isoforms MCT1, MCT2 and MCT4, which have been expressed in *Xenopus* oocytes, and all of which are also found in muscle tissue

(Bröer et al., 1998, 1999; Dimmer et al., 2000). In most species studied, MCT1 appears to be the main lactate carrier especially in oxidative muscle fibers, while MCT4 has been found to dominate in the glycolytic fibers (Wilson et al., 1998; Pilegaard et al., 1999; Bonen et al., 2000a). The expression of these two isoforms seems to be regulated by hormones and exercise (Juel, review 1998; Bonen et al., 2000a; Dubouchaud et al., 2000; Evertsen et al., 2001).

Although the exact mechanism of activation remains unknown, the MCTs, especially MCT1, appear to be upregulated by exercise (Bonen et al., 2000b). The upregulation of MCT4 by exercise is not as clear (Bonen et al., 2000b; Evertsen et al., 2001). The population of MCTs in the muscle of most species studied seems to consist solely of these 2 isoforms (Bonen, 2000). Pigs, however, seem to differ from this generalization, because only very small amounts of MCT1 are expressed in muscles. In porcine muscles, MCT2 together with MCT4 appear to be the major isoforms in both the oxidative muscles, such as *M. masseter*, and in the most glycolytic muscles, such as *M. longissimus dorsi* (Sepponen et al., 2002).

Oxidative muscle fibers consume large quantities of lactic acid, which is an excellent source of energy, as it contains more than 90% of the energy of glucose. It has been speculated that the major role of MCT1 is in this influx of lactate (Halestrap and Price, review 1999). During stressful situations, when ATP consumption in the cell exceeds the capacity of oxidative metabolism, lactate accumulates in the fiber, due to the shift towards anaerobic energy production. MCT4, which has a high Km for lactate, but also a high V_{max} value (Dimmer et al., 2000), is well suited to function in such conditions. MCT4 has been suggested to be the main transporter in lactate efflux. We have speculated that, because both the capillary and mitochondrial densities are low in porcine muscles, small amounts of lactate are continuously produced as a result of anaerobic glycolysis (Sepponen et al., 2002). In such cells, lactate concentration could be kept at a low level by the function of MCT2, which, when expressed in *Xenopus* oocytes (Bröer, 1999), has a very low K_m value for lactate. The driving force in this transport is the lactate concentration gradient across the membrane. After slaughter, however, a balance will eventually exist between the lactate concentrations within the fiber and the interstitial fluid, and the movement of lactate across cell membranes will begin to slow. Temperature efficiently increases the rate of this transport process, as demonstrated by Väihkönen et al. (2000) who studied lactate transport into equine red blood cells at a temperature range of 0 to 37ºC. Furthermore, the transporters are activated at low pH (Väihkönen et al., 2000).

The cloning and characterization of MCTs has broadened our horizons about the metabolism of lactic acid, but many questions still remain to be answered. For example, the role MCTs play in connection with stress susceptibility or meat quality is unknown.

The Buffering Capacity Reveals the Amount of Lactic Acid Needed

Muscles are well adapted to their function. The buffering capacity keeps their pH at the level that allows an effective function of vital enzymes. The buffering capacity is greater in muscles needed for fast, short-time bursts, and lower in muscles prone to long-lasting activity at a relatively low intensity. Therefore, as a rule, buffering capacity is greater in large glycolytic muscles with large-diameter muscle fibers and sparse capillarization. This suggests primarily anaerobic metabolism. Oxidative muscles with a small fiber diameter and high capillary density have a lower buffering capacity. The buffering capacity also changes within the physiological pH-range, depending on the method by which it has been determined, especially on the amount of water used in the determination. Kivikari (1996) found that with high water additions, the buffering capacity $\beta = dA/dpH$ (A= the amount of added acid or base) is at maximum (pH ca 7.0) ca 70 mmol H^+/pH*kg and at minimum (pH ca 5.5) ca 30. With lower water additions, the minimum and maximum tend to level off. Kivikari also suggests that the buffering capacity in an intact muscle is somewhat lower, ca 40 mmol H^+/pH*kg. Most studies have found the buffering maximum at pH 6.8 and the minimum at pH 5.0. There is a large increase in buffering capacity in pH values below 5 (Kivikari, 1996).

As mentioned above, the buffering capacity in most muscles is between 50 to 60 mmol H^+/pH*kg, but lower and higher values have been reported. The reported values in beef are slightly lower and less variable than in pork (can be compared only if determined in the same study). The post mortem reactions cause an increase in buffering capacity of about 8 to 10%. In practical terms and within the pH limits from 7 to 5.5, lactic acid is by far the most important free variable in muscle to determine the actual pH value at any given time.

In the physiological pH range myofibrillar proteins have a buffering capacity of about 20 to 25 mmol H^+/pH*kg. The soluble components influence the buffering capacity by about 10 (pH 5.5) to 30 mmol H^+/pH*kg (pH 6.8), carnosine and inorganic phosphate being the most important compounds (Kivikari, 1996). In summary, the buffering capacity value '50 mmol H^+/pH*kg' can be used as a "rule of thumb" in calculations to estimate the relationship of lactic acid content and pH value within the physiological range.

The Rate of ATP Consumption and Production

ATP is used for contraction of sarcomeres and other processes in muscle, maximally 200 to 300 mmol/kg*min

(Lister, 1988). In a resting muscle, the consumption is about 1/100 of the maximum, which is needed to maintain the ionic balance and provide the basic heat of the body (69% of the energy produced in the reaction ATP -> ADP + P is heat). During maximal exercise an animal can increase its oxygen consumption by more than 20 fold from the resting level. Because the energy consumption may increase 100 fold, the difference must come from some other source, which is the anaerobic energy production via glycogenolysis. It can be estimated that in an extreme case, the pH value of living muscle may fall to 6.2-6.4 in less than 30 seconds. (This is the case only in an anaerobic muscle, because dark muscles do not easily create such an extensive decrease in pH in vivo). This pH change would need about 40-60 mmol H^+/kg.

Immediately post mortem ATP is used in muscles for involuntary slow contractions, maintenance of membrane potential and especially to keep the calcium ions within the sarcoplasmic reticulum. Bendall (1979) showed that the ATP content remains at the normal level (8-10 mmol/kg) until half of the ultimate amount of lactic acid has been produced. Then the ATP content starts to decline, and when it reaches about 1 mmol/kg, there will be the onset of rigor mortis (which depend on the temperature of the muscle) (Honikel et al., 1983).

ATP, creatine phospate, and oxygen bound to myoglobin contribute to the ATP level during the immediate post mortem phase. When one molecule of lactic acid is produced, 1 or 1.5 molecules of ATP/lactate are also produced. Therefore, all the metabolic factors discussed here are inter-linked and should be handled simultaneously when discussing the pH of muscle and meat and its effects on meat quality.

Post Mortem Oxygen Utilization

The literature provides very variable data on the myoglobin contents of various meats. Generally, the content in light muscles ranges from 0.06 to 0.2 and in dark muscles 0.2 to 0.3 mmol/kg. In beef, the myoglobin content is higher and strongly age-dependent ranging from 0.1 to 1 mmol/kg. A full oxygen saturation of hemes would then mean equivalent concentrations of molecular oxygen. One 1 mmol oxygen produces 6 mmol ATP. Consequently, the oxygen stored in myoglobin, allows the production of 0.06*6=0.4 to 1*6=6 mmol ATP, depending on animal species, age and muscle type. In addition, meat contains minute amounts of blood. Depending on the degree of bleeding and the oxygen saturation of blood in various muscles, additional oxygen would be at disposal. No published data on this is available, but based on our preliminary data on hemoglobin hemi (not published) we estimate that the amount of available oxygen is not larger than the myoglobin reservoirs. We suggest that in total heme + oxygen contents, theoretically equaling about 15% of the post mortem production of ATP via glycolysis in beef and 1% in pork, and this difference might be one of the factors influencing the lower rate of pH decline in beef than in pork. The variation in the oxidative status of muscles at death and amount of residual blood may also have an influence on the onset and rate of glycolysis, respectively.

Creatine Phosphate is a Relevant Source of ATP

The content of creatine phosphate is *in vivo* 20-25 mmol/kg. This represents roughly 1/3 of the amount of ATP formed via glycogenolysis post mortem.

Concluding Remarks

Our present knowledge of the cardio-respiratory capacity of modern pig is insufficient. This, together with glycogen and the end product of its metabolism, lactate, has a strong influence on animal welfare and meat quality, which underlines the importance of further research in this field.

The pig has about 800-900 g of glycogen in its muscles and up to 150 g in the liver. During intense stress, the pig may readily utilize up to 1/6, i.e. ca 150 g, of this muscle glycogen pool very rapidly. The high proportion of white fibers and lowered capillary density in the modern pig, in comparison to wild pigs/primitive breeds in which the proportion of red and intermediate fibers as well as capillary density are higher, suggests that most of the glycogen is converted to lactate. At present, the quantitative data on the fate of lactate is limited. In living animals, lactate is ultimately used aerobically as a fuel (or converted to glucose in liver). The questions that warrant further quantitative studies include the following: Due to the high rate of glycogenolysis, how much lactate remains in the fibers and what is the capacity of MCTs to facilitate lactate efflux? Since white fibers minimally use lactic acid as a fuel, what is the proportion of lactate transported by MCTs to red fibers for further oxidation and how much lactate efflux occurs to the blood? What is the proportion of blood lactate that is transported to the liver for gluconeogenesis and how much is oxidized in other organs? The questions asked here may equally well apply to poultry, but beef animals are far more oxidative and are also lethargic, so that no substantial changes in glycogen levels in muscles would be expected during a short stress. Effects of alternative fuels, mainly lipids, are closely related to the carbohydrate metabolism and thus an important factor, but are out of the scope of this review.

Post mortem creatine phosphate as well as oxygen reservoirs may delay, but not replace, glycogenolysis. The welfare of the animal is connected to meat quality, because in a stressed animal both creatine phosphate and glycogen pools may already be depleted and oxygen reservoirs may be low in muscles that contain a high proportion of white fibers. Currently very little is known about how these differences may eventually influence the rate of post mortem sequences and therefore, meat quality. Further studies are needed to answer these questions.

Acknowledgements

The authors wish to thank Ms. Hilary A. Sepe for the language checking.

References

Adamo, K.B.; Graham, T.E. 1998. Comparisons of traditional measurements with macroglycogen and proglycogen analysis of muscle glycogen. Journal of Applied Physiology 84:908-913.

Bendall, J.R. 1979. Relations between muscle pH and important biochemical parameters during the post-mortem changes in mammalian muscles. Meat Science 3:143-157.

Bonen, A. 2000. Lactate transporters (MCT proteins) in heart and skeletal muscles. Medical Science and Sports Exercise 32:778-789.

Bonen, A.; Miskovic, D.; Tonouchi, M.; Lemieux, K.; Wilson, M.C.; Marette, A.; Halestrap, A.P. 2000a. Abundance and subcellular distribution of MCT1 and MCT4 in heart and fast-twitch skeletal muscles. American Journal of Physiology (Endocrinology and Metabolism) 278:E1067-E1077.

Bonen, A.; Tonouchi, M.; Miskovic, D.; Heddle, C.; Heikkila, J.J.; and Halestrap, A.P. 2000b. Isoform-specific regulation of the lactate transporters MCT1 and MCT4 by contractile activity. American Journal of Physiology (Endocrinology and Metababolism) 279:E1131-E1138.

Bröer, S.; Schneider, H.P.; Bröer, A.; Rahman, B.; Hamprecht, B.; Deitmer, J.W. 1998. Characterization of the monocarboxylate transporter 1 expressed in Xenopus laevis oocytes by changes in cytosolic pH. Biochemical Journal 333:167-174.

Bröer, S.; Bröer, A.; Schneider, H.P.; Stegen, C.; Halestrap, A.P.; Deitmer, J.W. 1999. Characterization of the high-affinity monocarboxylate transporter MCT2 in Xenopus laevis oocytes. Biochemical Journal 341:529-535.

Dawson, D.M.; Goodfriend, L.; Kaplan, N.O. 1964. Lactic dehydrogenases: Functions of the two types. Science 143:929-933.

Dimmer, K.S.; Friedrich, B.; Lang, F.; Deitmer, J.W.; Bröer, S. 2000. The low-affinity monocarboxylate transporter MCT4 is adapted to the export of lactate in highly glycolytic cells. Biochemical Journal 350:219-227.

Dubouchaud, H.; Butterfield, G.E.; Wolfel, E.E.; Bergman, B.C.; Brooks, G.A. 2000. Endurance training, expression, and physiology of LDH, MCT1 and MCT4 in human skeletal muscle. American Journal of Physiology (Endocrinology and Metabolism) 278:E571-E579.

Evertsen, F.; Medb•, J.I.; Bonen, A. 2001. Effect of training on muscle lactate transporters and lactate threshold of cross-country skiers. Acta Physiologica Scandinavica 173:195-205.

Fieldhause, B.; Masters, C.J. 1966. Developmental redistribution of porcine lactate dehydrogenase. Biochemica et Biophysica Acta 118:538-548.

Goldsmidt, E.; Sprang, S.; Fletterick, R. 1981. Structure of maltoheptose by difference Fourier methods and model for glycogen. Journal of Molecular Biology 156:411-427.

Graham, T.E.; Adamo, K.B.; Shearer, J.; Marchand, I.; Saltin, B. 2001. Pro- and macroglycogenolysis: relationships with exercise intensity and duration. Journal of Applied Physiology 90:873-879.

Gregory, N.G. Animal Welfare and Meat Science. CABI Publishing, New York. 1998.

Halestrap, A.P.; Price, N. T. 1999. The proton-linked monocarboxylate transporter (MCT) family: structure, function and regulation. Biochemical Journal 343:281-299.

Hamm. R.; El-Baldawi, A.A. 1991. Aktivität und subzellulare Verteilung der Laktatdehydrogenase in heller and dunkler Musculatur von Rind and Schwein. Fleischwirtschaft 71:813-816.

Honikel, K.O.; Ronchales, P.; Hamm, R. 1983. The influence of temperature on shortening and rigor onset in beef muscle. Meat Science 8:221-241.

Hyyppä, S.; Pösö, A.R. 1998. Fluid, electrolyte, and acid-base responses to exercise in racehorses. Veterinary Clinics of North America: Equine Practice 14:121-136.

Immonen, K. 2000. Bovine muscle glycogen concentration in relation to diet, slaughter and ultimate beef quality. PhD thesis. University of Helsinki, Department of Food Technology Series 1203.

Immonen, K.; Puolanne, E. 2000. Variation of residual glycogen-glukose concentration at ultimate pH values below 5.75. Meat Science 55:279-283.

Juel, C. 1997. Lactate-proton cotransport in skeletal muscle. Physiologica Reviews 77:321-358.

Juel, C. 1998. Muscle pH regulation: role of training. Acta Physiologica Scandinavica 162:359-366.

Juel, C.; Halestrap, A.P. 1999. Lactate transport in skeletal muscle - role and regulation of the monocarboxylate transporter. Journal of Physiology 517:633-642.

Kivikari, R. 1995. The buffering capacity of meat. PhD thesis. University of Helsinki, Department of Food Technology Series 1048. 131 p.

Lacourt, A.; Tarrant, P.V. Selective glycogen depletion and recovery in skeletal muscle fiber types of young bulls. The Problem of Dark-cutting in Beef. Hood, D.D.; Tarrant, P.V. Martinus Nijhoff Publishers. The Hague, Boston, London. 1980, 417-429.

Lawrie, R.A. Meat Science. 6th ed. Woodhead Publishing Ltd. Cambridge. 1998, 336 p.

Lister, D. Muscle metabolism and animal physiology in the dark cutting condition. Dark cutting in cattle and sheep. Fabiansson, S.U.; Shorthose, W.R.; Warner, R.D. Australian Meat & Live-stock Research & Development Corporation. 1989, 19-25.

Meléndez-Hevia, E.; Waddel, T.G.; Shelton, E.D. 1993. Optimization of molecular design in the evolution of metabolism: the glycogen molecule. Biochemical Journal 295:477-483.

Nelson, T.E.; White, R.C.; Gillard, B.K. 1979. American Chemistry Symposium Series 88:131-162.

Pilegaard, H.; Terzis, G.; Halestrap, A.P.; Juel, C. 1999 Distribution of the lactate/H^+ transporter isoforms MCT1 and MCT4 in human skeletal muscle. American Journal of Physiology (Endocrinology and Metabolism) 276:E843-E848.

Pösö, A.R. 2002. Monocarboxylate transporters and lactate metabolism in equine athletes. Acta Veterinaria Scandinavica 43:63-74.

Rosenvold, K. 2002. Strategic feeding - a tool in the control of technological pork quality. PhD thesis. Swedish University of Agricultural Sciences, Agraria 314. 59 p.

Rosenvold, K.; Essén-Gustafsson, B.; Andersen, H.J. 2002. Dietary manipulation of pro-and macroglycogen in porcine skeletal muscle and its effects on post-slaughter glycolysis. Journal of Applied Physiology. Submitted 2002.

Ryman, B.E.; Whelan, W.J. 1971. New aspects of glycogen metabolism. Advances in Enzymology and Related Areas in Molecular Biology 34:285-443.

Schäfer, A.; Rosenvold, K.; Purslow, P.P.; Andersen, H.J.; Henckel, P. 2002. Physiological and structural events post mortem of importance for drip loss in pork. Meat Science 61:355-366.

Sepponen, K.; Koho, N.; Puolanne, E.; Ruusunen, M.; Pösö, A.R. Distribution of monocarboxylate transporter isoforms MCT1, MCT2 and MCT4 in porcine muscle. A manuscript. 2002.

Suuronen, T. 1996. The relationship of oxidative and glycolytic capacity of *Longissimus dorsi* muscle to meat quality when different pig breed and crossbreeds are compared. PhD thesis. Biological Research Reports of University of Jyväskylä 46. 112 p.