CARBOHYDRATE METABOLISM IN MEAT ANIMALS

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Introduction

This review deals with the perimortem oxidative/glycolytic capacity of meat animals, especially in muscle tissue. We discuss carbohydrate metabolism focusing on the relationships between the pre-mortem and post-mortem phenomena. The nutritional and hormonal regulation of energy metabolism in skeletal muscles of meat animals has recently been thoroughly reviewed e.g. by Hocquette, Ortigues-Marty, Pethick, Herpin & Fernanadez (1998) and will not be treated here. The subject discussed here has also been presented by the authors of the current review also elsewhere, at Reciprocal Meat Conference (Puolanne, Pösö, Ruusunen, Sepponen & Kylä-Puhju, 2002)

Carbohydrates are essential in the conversion of muscle to high quality meat, but in live animals they are stored in the muscles to provide the animal with a rapidly available source of energy. In "fight-or-flight" situations the muscles have to produce energy at the highest possible rate and even in animals with high aerobic capacity part of this energy production will involve anaerobic pathways and accumulation lactic acid, which is the end product of anaerobic glycogen breakdown. Within the animal kingdom horses have a high oxidative capacity, as indicated by maximal oxygen uptake up to 160 ml/kg body weight per min (Tyler, Golland, Evans, Hodgson & Rose, 1996; Langsetmo, Weigle, Fedde, Erickson, Barstow & Poole, 1997). This is more than twice the value measured in human athletes and about three times that in cattle (Kayar *et al*., 1994) and almost 6 times the value measured in pigs (Perez-de-Sá, Cunha-Goncalves, Schou, Jonmarker & Werner, 2003). Theoretically, if it is assumed that during intense exercise 90% of oxygen is consumed by exercising muscles and that approximately 40% of the body weight is muscle, it can be calculated that with the reported maximal oxygen uptake of horses, aerobic ATP production in the equine muscle may approach 2 µmol/g per s and a similar calculation for pigs gives a value of 0.35 µmol/g per s. Although this calculation underestimates the true production of ATP in working muscles, because all muscles are not equally active as assumed in the calculation above, the values are well below the approximated maximal ATP demand, the calculated value of which for human muscle is about 3 to 5 µmol/g per s (Newsholme & Leech, review 1986). The difference between the demand and aerobic production has to be met by anaerobic glycolysis, the end product of which is lactic acid. This is possible, because the capacity of glycolytic enzymes, at least in equine and porcine muscles, is high enough to support the ATP production that allows maximal rate of ATP consumption (Essén-Gustavsson, Karlström & Lindholm, 1984; Newsholme & Leech, review 1986; Cutmore, Snow & Newsholme, 1993; Karlström, 1995).

The aerobic capacity is determined by the capacity of cardiovascular system and muscle related factors, such as fibre composition and mitochondrial density. On the cardiovascular side, the cardiac output and capillary density in muscles determine the availability of oxygen and inside the muscle the mitochondrial density is a key factor in determining the rate of oxygen utilization. In meat animals the aerobic capacity decreases in the following order cattle > pig > poultry. The size of the heart in beef cattle has been reported to be approximately 0.44% of live weight (Sainz & Bentley, 1997) and in pigs 0.33% (Ruusunen & Puolanne, unpublished), and even greater difference is seen in the capillary density. In the longissimus muscle of cattle the capillary density is approximately 450/mm^2 (Karlström, Essén-Gustavsson & Lindholm, 1994), but in pigs the value is only 150/mm^2 (Ruusunen & Puolanne, 2004). It is generally accepted that the blood flow into skeletal muscle at rest is about 20% of cardiac output, but it may increase to up to 80% of cardiac output

during intense exercise. When this is combined with the increase in cardiac output, the actual increase in blood flow may be 15-fold (or in the case of athletic animals such as horses even more). In such situations the capillary density of the muscles may play an important role. Higher capillary density in the muscles of cattle may result in a better supply of oxygen and efflux of end products of metabolism than is possible in porcine muscles. Thus it can be speculated that during stress both the production of lactic acid as well as accumulation of lactate will be greater in the muscles of pigs than of cattle.

The amount of glycogen in muscles varies from species to species. In the longissimus muscle of cattle the measured concentrations vary between 60-100 mmol/kg (wet) tissue (Immonen, Kauffman, Schaefer & Puolanne, 2000a), in pigs values are around 85 mmol/kg (Rosenvold, Laerke, Jensen, Karlsson, Lundström & Andersen, 2001), but in horses glycogen concentration is usually 1.5 times the concentration measured in cattle (Lindholm, Bjerneld & Saltin, 1974; Hyyppä, Räsänen & Pösö, 1995).

The Glycogen Molecule Complex

The glycogen molecule contains a core protein, glycogenin (MW 37,300 Da), which has enzyme-like properties and supports and catalyses the synthesis of an 8 glucosyl-unit-long primer, necessary for the activity of glycogen synthase (Nelson & Cox, review 2005). 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 $1+2^{12}$, or about 4,100. A glycogen molecule with the molecular weight of nine to ten million Da contains about 55,000 glucose residues, the diameter of the molecule is close to 40 nm and the number of available non-reducing ends is approximately 2,100. The full outermost tier contains always roughly 50% of the total carbohydrate of the molecule. 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 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,Waddel & Shelton, 1993).

Proglycogen and Macroglycogen

The glycogen molecules exist in two forms: proglycogen (PG) and macroglycogen (MG) (Adamo and Graham, 1998). 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 glycogen, while in stressed muscle with less glycogen, its proportion is about 90% (Adamo & Graham, 1993). The relative amount of the acid soluble macroglycogen (0.4% protein, MW 10^7 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 diet (Rosenvold, Essén-Gustafsson & Andersen, 2002). It remains to be seen, how much of glycogen in the form of acid-insoluble PG has not been taken into account in the earlier published studies in which the glycogen has been extracted by acid.

As each chain, also in PG, has 13 glucosyl residues (MW: 13*162 = 2106/chain) it can be calculated that the PG molecules have 6 (MW ca 300,000 including glycogenin) to 7 tiers (MW ca 575,000). In theory, 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 are practically the same. In comparison, in a MG molecule, the number of tiers with a molecular weight of 10^7 Da is 13 and the number of non-reducing ends approximately 2,100.

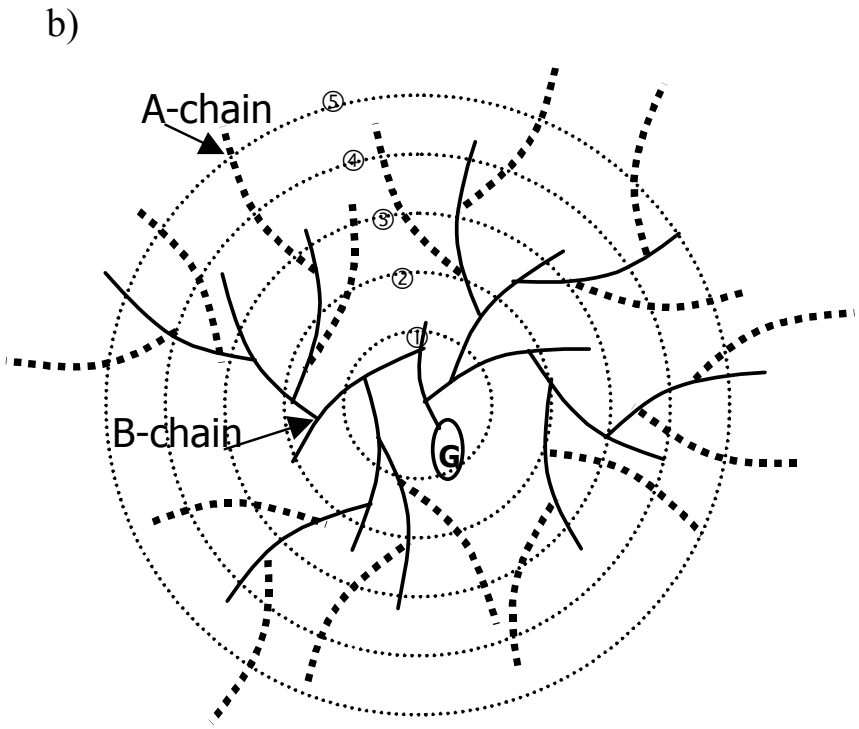

Figure 1. Scheme showing the structure of the glycogen molecule as stated in Whelan's model (Adopted from Immonen, 2000)

Glycogen breakdown: phosphorylase

In muscle, the main sources of glucose are blood glucose (from liver glycogen) and glycogen stored in muscles (Hocquette *et al*., review 1998). During stress, hormonal mechanisms are important for the mobilization and utilization of carbohydrates. In muscle, catecholamines, released from sympathetic neurons and adrenal medulla, induce rapid degradation of glycogen through the activation of glycogen phosphorylase and the inhibition of glycogen synthesis. In addition phosphorylase is under allosteric (AMP and/or Ca^{++} activated) control. Activated phosphorylase cleaves glucosyl units as glucose-1-P from a glycogen molecule at an extremely high speed. The reaction cascade is explicitly described in every textbook of biochemistry and therefore, will not be presented here. The sequence is blocked when the influx of blood glucose meets the demand of energy, and when the enzymes of glycogen synthesis are activated (Nelson & Cox, review 2005).

The catalytic subunit of a glycogen phosphorylase dimer (or tetrame) is bound to glycogen, whereas the other subunit regulates the function of the enzyme (Goldsmith, Sprang & Fletterick, 1982). The phosphorylase content is very high, about 2% of the total protein in muscles (Ryman & Whelan, 1971). Glucose residues from the non-reducing end of the uppermost, unbranched A-chains, are cleaved until the fourth glucosyl unit from the branching point. Phosphorylase cannot cleave glucosyl units from the branched B-chain that is too short. Thus, theoretically 34.6% of the units of the outermost tier are available to phosphorylase, i.e. in the case of 100 mmol/kg glycogen (as glucosyl units) in muscle about 35 mmol/kg glucose-1-P will be produced. This results in the formation of 70 mmol/kg lactate (and 105 mmol/kg ATP), if all energy were 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 release of glucosyl units of the lowermost tiers, when the glycogen content is low (Meléndes-Hevia *et al.*, 1993).

Recently it has been shown in humans and horses that MG is preferentially used in long-lasting aerobic stress (Asp *et al*., 1999; Essén-Gustavsson & Jensen-Waern 2002), but PG is utilized more during anaerobic stress (Graham, Adamo, Shearer, Marchand & Saltin, 2001; Bröjer, Jonasson, Schuback & Essén-Gustavsson 2002) as well as in pigs post mortem (Rosenvold *et al.*, 2002). 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/or by AMP (Nelson & Cox, review 2005).

Glycogen breakdown: 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 (GDE) 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 enables the continuation of the action of phosphorylase (Nelson & Cox, review 2005). 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 12 glucose residues as glucose-1-P, the debranching enzyme cleaves only one as glucose (Meléndes-Hevia *et al.*, 1993). There is some evidence that the activity of the debranching enzyme limits the rate of glycogen breakdown in situations where more than the uppermost layer is to be used (Nelson, White & Gillard, 1969). It can also be anticipated that because of the activity of phosphorylase on glycogen molecules with low number of non-reducing ends is reduced due to spatial reasons, 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, non-stressed animals the residual glycogen may be much higher, up to 80 mmol/kg, even when the ultimate pH is 5.5 (Immonen & Puolanne, 2000).

Kylä-Puhju, Ruusunen & Puolanne (2004b, c) were able to show that the activity of GDE is not particularly affected by the pH in the pH range 5.5–7. But, on the contrary, temperature fall had a very strong effect on the activity, which was close to zero at temperatures below 15 ºC. This may indicate that the positive effects of fast chilling of porcine PSE carcasses (Honikel, review 1984; Offer & Knight, review 1988; Schäfer, Rosenvold, Purslow, Andersen & Henckel, 2002) may be partly based on the elimination of a source of rapidly fermentable glucose due to inactivation of GDE. In the case of beef, where the rate of glycogenolysis as well as carcass chilling are much slower, the lowering of the temperature may block the GDE activity. However, it is not known, how long the A-chains are at death or how long it takes before 15 ºC is reached. Thus there could be variable amounts of glucose-1-P available. The lactate derived from glucose is effective at lowering the pH. Although the buffering capacity, depending on animal species and type of muscle, is from 40 to 60 mmol/(kg∗pH), already small decreases in pH, e.g. 0.2–0.3 pH units, that can be a result from the accumulation of 10 to 20 mmol/kg of lactic acid derived from 5 to 10 mmol of glucose equivalents, are relevant and have a marked effect on water-holding, colour and tenderness. In addition, small differences in the rate of post mortem pH decline have huge effects on the traits mentioned above.

A limited meta analysis of the data EU Concerted Action CT94-1881 coordinated by Joseph and Troy (1998, see the references in the Proceedings; numbers refer to the papers in the Tomes 1, 2 and 3) revealed that in most cases fast chilling produced beef where the pH_{24} was ca. 0.1–3 units higher than by conventional cooling (e.g. Sheridan, McGeehin & Butler, 1.10; Ronchales & Beltram, 2.23; Beltram & Ronchales, 2.24; Beltran, Jaime & Ronchales, 2.5; Ronchales, Jaime & Beltràn, 2.8; Beltran, Tomas & Ronchales, 3.14; Tomas II176, Steen, Claeys & Demeyer, 3.6, Claeys, Demeyer & Van de Voorde, 3.12; Richardson & Perry, 3.15), although in several studies this was not found, or it was even the opposite (e.g. Klont & Eikelenboom, 1.11; Montanac-Bissay & Lepetit, 2.10; Troy & Vidal, 3.4; Taylor, Richardson & Perry, 3.10; O'Mahony, Joseph, McKenna, 3.17; Trevisani, Loshi & Severini, 3.19; Smulders, van Laack & Hofbauer, 3.20). Sometimes there was only an initial reduction in pH fall suggesting that the remaining activity of phosphorylase and GDE could be enough when the time available is long enough (White, Troy & McKenna, 1.13; Steen, Claeys & Demeyer, 3.6; Trevisani, Loshi & Severini, 3.11). This, however, needs further research, and a similar study on electrically stimulated carcasses should be done. It should also be pointed out that pH and temperature change rates differ much in carcasses, which makes the scrutiny of these aspect difficult for practical considerations (Puolanne & Ruusunen, 1998).

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 & Cox, review 2005). In muscles, as in other tissues, lactate dehydogenases 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 H_4 (LDH-1) and M_4 (LDH-5) (Dawson, Goodfriend & Kaplan, 1964; Fieldhouser & Masters, 1966). LDH-5 has a high K_m 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. Although the M-type dominates in dark and white muscles, the dark muscles contain more H-type activity when compared to light muscles. For example in infraspinatus muscle about 30% of the chains in LDH isoenzymes are H-type while the percentage in longissimus muscle is only 4 (Ruusunen, Sepponen, Puolanne & Pösö, unpublished). Porcine muscles have an exceptionally high LDH activity (Hamm & El-Badawi, 1991; Suuronen, 1995). 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 efficiently in the heart and dark (oxidative) muscles than in light (glycolytic) muscles.

The Formation of Lactic Acid

In the muscle fibres of a living animal, small amounts of lactic acid is produced almost constantly. The activation of glycogen phosphorylase during stress increases the accumulation of lactic acid and consequently that of protons causing fatigue, and ultimately, pain and distress (Gregory, review 1998). The acidification of the muscles leads to attenuation of the rate of glycolysis, because protons inhibit the activities of glycogen phosphorylase and phosphofructokinase and thus energy production from carbohydrates (Fitts, review 1994). Protons may also inhibit the function of myosin ATPase (Fitts, review 1994; Schiaffino & Reggiani, review 1996) and thus impair muscle contractions. Furthermore, both the uptake and release of calcium ions from the sarcoplasmic reticulum are disturbed due to acidification (Westerblad, Lee, Lännergren, & Allen, review 1991; Williams & Klug, review 1995). In fatigued muscle the pH may be as low as 6.5 to 6.3 (Lowell, Reid & Rose, 1987; Juel, review 1996), but this value is significantly higher than the ultimate pH in muscles after slaughter that is usually around 5.4–5.8

When a glucose molecule 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 & Cox, review 2005). Lactic acid formed is either converted back to pyruvic acid to be used oxidatively via the tricarboxylate acid cycle, or when there is a lack of oxygen and/or mitochondria, transported out of the fibre.

When pH in muscles is 6.2, the concentration of lactic acid in the muscle fibres must be about 50 to 60 mmol/kg. In a 100-kg live weight modern lean pig (47 kg muscle tissue) this would mean 47 kg*55 mmol/kg*90 mg/mmol lactic acid, i.e. ca 230 g. Whether or not this overestimates the amount of lactic acid formed (all the muscles do not produce simultaneously this maximal amount of lactic acid), it means that after a stress situation this amount should be utilised in the adjacent fibres or released to blood. For aerobic tissues lactate is an excellent source of energy, because it contains most of the energy of glucose. In live animals all the lactate formed during stressful situations is either oxidized in heart, liver and aerobic muscles or converted back to glucose in liver (Pösö, 2002). It is also generally accepted that, within the muscle tissue, some lactate may be transported via the interstitial fluid from white, glycolytic, fibres to red fibres (type 1 and IIA) which have higher mitochondrial density. It can be speculated that in domestic pigs this

phenomenon is of minor importance, because the percentage of red fibres is small in muscles such as *M. longissimus, gluteus* and *semimembranosus* (Ruusunen 1994; Karlström, 1995; Ruusunen & Puolanne 2004). Without uptake of lactic acid into tissues for oxidation, only small amounts can be released into blood. Lactic acid concentration in blood may vary between 5–25 mmol/l, which in a 100-kg pig corresponds to about 10 g. The imbalance between formation and transport capacity of blood is non-physiological, and problems caused by the low oxidative capacity of modern pigs and poultry can be expected to rise if the increase in percentage of glycolytic muscle tissue will continue, due to the better feed conversion rate of glycolytic fibres (Hocquette *et al*., review 1998). The problem is not as severe in ruminants, which are lethargic and have higher oxidative capacity than pigs. When they are stressed and catecholamines evoke glycogenolysis, a large part of pyruvic acid will be utilised aerobically, and therefore large variation in pH in living muscle will not be probable.

The Buffering Capacity

Muscles are well adapted to their function. The buffering capacity keeps their pH at a level that allows an effective function of vital enzymes. The buffering capacity is greater in muscles needed for fast, bursts of short duration, and lower in muscles prone to long-lasting activity at relatively low intensity. Therefore, as a general rule, buffering capacity is greater in large glycolytic muscles with large-diameter muscle fibres and sparse capillarization. This suggests primarily anaerobic metabolism. Oxidative muscles with a small fibre diameter and high capillary density have a lower buffering capacity.

At rest the pH of porcine longissimus muscle varies from 7 to 7.2 (Tarrant, McLoughlin & Harrington, 1972; Kivikari, 1996; Kylä-Puhju, Ruusunen, Kivikari & Puolanne, 2004a). Because oxidative pathways produce carbon dioxide, protons are continuously formed in muscle cells according to the following equation:

$$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow H^+ + HCO^-_3.$$

Large part of the carbon dioxide diffuses from the muscle into red blood cells and protons that are formed there are buffered by hemoglobin. In the muscle cells the formation of protons activates Na^+/H^+ exchange protein that carries one H^+ out from the cell and simultaneously one Na^+ into the cell (Madshus, 1988). This carrier is driven by the Na+ gradient (Madshus, 1988). There are no studies on the activity of Na^+/H^+ exchange in the muscles of meat animals, but in human muscle this carrier plays the key role in the regulation of cell pH at rest (Juel, Lundby, Sander, Calbet & van Hall, 2003).

A major difference in buffers between muscle and meat is the bicarbonate system that is very efficient in the live animals, but does not have any major role post mortem. Low pH acts as a signal to peripheral chemoreceptors and causes an increase in the rate and intensity of respiration. In lungs the reactions that in the tissues lead to formation of protons proceed in the opposite direction and the carbon dioxide is subsequently exhaled:

$$H^+ + HCO^-_3 \rightarrow H_2CO_3 \rightarrow CO_2 + H_2O\ .$$

Together these two reactions show that for each mole of CO_2 exhaled through the lungs one mole of protons is neutralized. Furthermore, the kidneys participate in the compensation by increasing the reabsorption of bicarbonate and also synthesizing it. In live animals lactate is also used in tissues, such as heart and liver, where metabolism is aerobic. Bicarbonate buffering system and the use of lactic acid in oxidative tissues prevent the drop of pH in live animals to such a low values seen post mortem, but after slaughter, the two mechanisms above have no bearing.

Most studies have found the buffering maximum at pH 6.8 and the minimum at pH 5.0. If pH is further lowered to values below 5, there is again a large increase in buffering capacity (Kivikari, review 1996). The latter increase is due to accumulation of lactic acid (Kivikari, review 1996). In addition to pH, the buffering capacity of post mortem muscles also varies according to the method used for its determination. Especially important is the amount of water used in the titration method determination. Kivikari (1996) and Puolanne & Kivikari (2000) found that with high water additions, the buffering capacity $\beta = dA/dpH$ (A= the amount of

added acid or base) has the maximum of ca 70 mmol H^+/(pH*kg) at the pH of ca 7.0, and the minimum of ca 30 mmol H^+/(pH*kg) at the pH of ca 5.5. This is in accordance with many other studies. With lower water additions, the minimum and maximum tend to level off. Kivikari (1996) also suggests that the buffering capacity in an intact muscle is somewhat lower, ca 40 mmol H^+/(pH*kg).

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, review 1996). Because post mortem reactions cause and increase in buffering capacity of about 8 to 10%, the buffering capacity in most post mortem muscles is between 40 to 60 mmol H^+/(pH*kg), but both 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). 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. The low pKa value of lactic acid (3.86) means that at the physiological pH range it is almost completely dissociated to a lactate anion and a proton. Without buffers, accumulation of lactic acid would mean a very rapid drop of pH. For example, Lindinger, McKelvie and Heigenhauser (review 1995) have estimated that without buffers, human arterial blood lactate concentrations would be 162 mmol/l and that plasma pH would drop almost to 1 after four 30-second bouts of high-intensity exercise. Kivikari (1996) concludes that the buffering capacity value of '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.

We have done a meta-analysis on literature data on relationships between glycogen contents, lactic acid contents, pH-values and buffering capacities in *M. longissimus dorsi* (Puolanne, Yli-Hemminki & Kylä-Puhju, 2004). The pH value of 7.2 was used as the zero lactate value. Based on the meta-analysis we found that the best fit between lactic acid, pH value and buffering capacity can be found when using buffering capacity value of 62 mmol H^+/(pH*kg). This was markedly higher than the 49 mmol H^+/(pH*kg) found by Kylä-Puhju *et al.* (2004a), who calculated the values on the basis of pH-lactic acid data of muscle analyses. These findings show once again that the buffering capacities depend on the methods by which they are determined, and much still remains to be studied.

Monocarboxylate transporters

When the rate of the formation of protons is high in living animals, buffering capacity alone is not sufficient to maintain pH homeostasis. In addition to Na^+/H^+ exchange, low pH activates also another proton carrier, the monocarboxylate transporter (MCT). MCTs form a transmembrane protein family, which cotransport a proton together with a lactate anion. In addition to lactate, MCTs also facilitate the transport of other monocarboxylate anions, such as short chain fatty acids (SCFA) and ketone bodies, through cell membranes (Halestrap & Meredith, review 2004). MCT-proteins are also involved in the absorption and delivery of monocarboxylic drugs, such as salicylic acid and simvastatin (Enerson & Drewes, review 2003). Recently isoform 8 (MCT8) was shown to transport thyroid hormones into cells (Friesema, Ganguly, Abdalla, Manning Fox, Halestrap & Visser, 2003).

Monocarboxylate transporter family is comprised of at least 14 members, which differ in their tissue and species specificity as well as in substrate affinity (Halestrap & Price, review 1999; Halestrap & Meredith review 2004). Among the best characterized are isoforms 1-4, but the role and characteristics of several isoforms are not known. Very little is also known about the regulation of MCT genes. Leptin has been shown to upregulate MCT1 mediated butyrate uptake in colonocytes (Buyse, Sitaraman, Liu, Bado, & Merlin, 2002) and erythropoietin increases the amount of MCT1 protein in erythrocyte membranes (Connes , Caillaud, Mercier, Bouix & Casties, 2004).

The topology of MCTs shows that they have 12 transmembrane domains with both C- and N-terminae in the cell (Halestrap & Price, review 1999; Halestrap & Meredith, review 2004). At least 3 isoforms, MCT1, MCT2 and MCT4, are expressed in skeletal muscle tissue (Juel & Halestrap, review 1999). MCT1 is a 53 kDa protein which, when expressed in *Xenopus* oocytes, has a K_m for lactate of 3-5 mM. Similarly expressed MCT2 has low K_m for lactate, 0.7 mM, while MCT4 expressed especially in glycolytic tissues has a much

lower affinity for lactate (K_m 28 mM) (Halestrap & Price , review 1999; Halestrap & Meredith, review 2004). The apparent V_{max} values for the influx of lactate into *Xenopus* oocytes are rather similar in MCT1 and MCT4, which is about 17-18 times that of MCT2 (Bröer, Bröer, Schneider, Stegen & Halestrap, 1999; Dimmer, Friedrich, Lang, Deitmer & Bröer, 2000). MCT1 and MCT4, but not MCT2, need a chaperone protein CD147 (basigin, EMMPRIN, neurothelin) to translocate and obtain active conformation in the cell membrane where they form a complex that consists of two MCT molecules and two CD147 molecules (Halestrap & Meredith, review 2004).

In most species including beef cattle (Koho & Pösö, unpublished results), MCT isoforms 1 and 4 are expressed in muscle tissue (Halestrap & Meredith, review 2004). In glycolytic muscle fibres both MCT1 and MCT4 facilitate the efflux of lactate when the rate of its production is high, whereas in oxidative fibres (type I) the dominant isoform is MCT1 which facilitates either influx or efflux of lactate depending on the prevailing electrochemical gradient of lactate (Halestrap & Meredith, review 2004).

In the muscles of domestic pig (Sepponen, Koho, Puolanne, Ruusunen & Pösö, 2003) and also those of wild boar (Sepponen, Ruusunen, Puolanne & Pösö, unpublished) only traces of MCT1 can be found. The low amount of MCT1 is not due to the lack of the MCT1 gene, since in porcine heart and intestines it is expressed (Sepponen, Ruusunen & Pösö, manuscript in preparation). Instead, the pig muscles express MCT2, which has high affinity for lactate (Halestrap & Meredith, review 2004). We have speculated that MCT2 is needed, because of the anaerobic nature of the porcine muscle fibres, which may involve continuous production of small amounts of lactate (Sepponen *et al*., 2003). The role of MCT2 would thus be to facilitate the efflux of lactate from cells where mitochondrial density is low. Although our knowledge on monocarboxylate transporters is increasing, there is no data available on their role post mortem.

The Rate of ATP Consumption and Production

ATP is used for contraction of sarcomeres and other processes in muscle, maximally 3 to 5 μmol/(g*s) (Lister, 1988). In a resting muscle, the consumption is about 1/100 of the maximum (0.03–0.05 μmol/(g*s)) 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 + heat). It can be calculated that during maximal exercise an animal can increase its oxygen muscle consumption by more than 20 to 40 fold from the resting level. The increase is based on increase in cardiac output, increased relative amount of circulation through muscles, and increased release of oxygen from blood to muscles. 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 in dark muscles do not easily show such an extensive decrease in pH *in vivo*. This pH change would need about 40–60 mmol H^+/kg.

Post-mortem the rate of pH fall may be in beef and sheep 0.005 and in porcine muscle 0.01 pH units/minute (Pearson & Young, 1989) or in PSE pig 0.02 (extreme PSE pig 0.1), in normal turkey 0.03 and in PSE turkey 0.06 pH units/minute (Sosnicki, Creaser, Piertrzak, Pospiech & Santé, review 1998). From that it can be estimated, using the buffering capacity of 60 mmol H^+/(pH*kg) and ATP/lactic acid molar rate of 1.5/1, that the ATP production varies in post mortem muscles of different species from 0.008 (beef and sheep), 0.015–0.03 (pig – PSE pig) (0.15 extreme PSE pig) to 0.05–0.09 μmol/(g*s) (turkey – PSE turkey) which is well in accordance with the values estimated in living resting muscle. It is not fully known, what are all the relevant reactions where ATP is consumed post mortem and why there are differences like between normal muscle – PSE muscle.

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, Ronchales & Hamm, 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

In a living animal, the partial pressure of oxygen in venous blood from resting muscles is about 40 torr, with oxygen saturation of haemoglobin of about 75% and in intensively working muscle the respective values are 20 torr or less and about 38%. If the physical stress further increases, myoglobin which is saturated up to 90%, starts to provide oxygen. When oxygen derived from myoglobin has been utilised, the oxygen pressure is approaching zero, and the fibre will be produce energy anaerobically via glycogen breakdown as described above. Consequently, both routes of ATP production are active simultaneously. 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. The full oxygen saturation of heme would then mean equivalent concentration of molecular oxygen. One 1 mmol oxygen enables an aerobic production of 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 available. 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 no larger than the myoglobin reservoirs. The amount of oxygen in sarcoplasma is also very small.

The amount of ATP produced by the formation of about 80 mmol/kg lactic acid is 120 mmol/kg (the post mortem formation of lactic acid molecule results in 1.5 molecules ATP). Consequently, we suggest that oxygen in total heme content theoretically equals about 5% of the post mortem production of ATP via glycolysis in beef and less than 1% in pork. This difference might be one of the factors causing the lower initial 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

The content of creatine phosphate is *in vivo* 20–25 mmol/kg. The production of ATP through the reaction catalyzed by creatine phosphokinase represents roughly 1/5 of the amount of ATP formed via glycogenolysis post mortem. When CP is broken to C and inorganic P, one proton is bound, which means an increase in pH as follows (Hyyppä & Pösö, review 1998).

$$CP^{2-} + ADP^{3-} + H^{+} \rightarrow ATP^{4-} + H_2O + C$$

This might, explain the initial increase in pH that is sometimes seen to take place during the slaughter process (unpublished observations). Taking the direct formation of ATP and also the elimination of protons into the account it can be calculated that this means a transient initial “buffering of the pH fall of almost one pH unit” compared to the situation when all creatine phosphate resources have been exhausted, and ATP will immediately be formed directly via glycolysis. Later ATP will, however, be formed by glycolysis anyway, and only the effect of the utilisation of protons (see equation above) will remain. Therefore, the content of creatine phosphate at the moment of slaughtering could be of importance. This has been recently thoroughly discussed by the similar way by Henkel, Karlsson, Jensen, Oksbjerg & Pedersen (2002).

Concluding Remarks

In living animals, the accumulation of lactic acid causes pain and distress and can result in a reduction in life quality. Our present knowledge of the cardio-respiratory capacity of meat animals, especially modern pigs and poultry is insufficient. This, together with glycogen and the anaerobic end product of its metabolism, lactate, has a strong influence on animal welfare and meat quality, which underlines the importance of further research on this field.

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