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Combined High-Field ^{13}C CP MAS NMR and Low-Field NMR Relaxation Measurements on Post Mortem Porcine Muscles

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Changes in postmortem muscle characteristics are investigated in muscles from eight pigs exposed to different combinations of preslaughter stress (exercise on treadmill) and stunning method (CO_2 vs electrical stunning). Solid-state ^{13}C cross-polarization (CP) magic-angle spinning (MAS) NMR experiments are carried out on a total of 16 rapidly frozen *M. longissimus* muscle biopsies taken in vivo the day before slaughter and at 45 min postmortem. Simultaneously, low-field NMR T_2 relaxation time measurements are carried out on samples from *M. longissimus.* Glycogen and lactate are estimated from the ^{13}C CP MAS spectra, and correlations of $r = 0.89$ and $r = 0.70$, respectively, to subsequent biochemical determinations using partial least squares regression (PLSR) are established. Moreover, PLSR reveals that, besides the 72 ppm signal (carbons in glycogen), a signal around 38 ppm, which increases concomitantly with lactate, is also significantly correlated to changes in glycogen/lactate. With the assumption that the 38 ppm signal reflects CH_2 in phosphocreatine/creatine, altered mobility of creatine as a result of dephosphorylation is indicated. Finally, PLSR on the 45 min ^{13}C CP MAS spectra also reveals correlation ($r = 0.54$) to the slowest relaxing T_2 population (50 min postmortem), known to reflect extra-myofibrillar water. Subsequently, evaluation of the loading plot in the PLSR analysis reveals that the correlation exclusively is associated to the 52 ppm resonance intensity. With the assumption that this resonance reflects methyl groups in choline/phosphatidyl choline, the intensity changes in the 52 ppm resonance imply alterations in membrane properties. Accordingly, the data indicate a relationship between membrane properties and the amount of water being expelled from muscle cells postmortem, which supports the hypothesis that disruption of membranes is implicated in the postmortem mobilization of muscle water.

KEYWORDS: Membrane destabilization, glycogen, lactate, creatine, magic angle spinning, muscle metabolism, carbon, T_2 relaxation

INTRODUCTION

During the postmortem period, several biochemical and biophysical processes are taking place in muscles, and these processes are known to be decisive for meat quality development, in particular the ability of the meat to retain water, referred to as water-holding capacity (WHC). Microscopic studies have shown a formation of extra-myofibrillar water channels postmortem, which is meant to be decisive for the WHC of meat (*1*, *2*). This redistribution of muscle water postmortem has recently been confirmed by NMR transverse (T_2) relaxation time measurements, which have revealed a redistribution of water with a resultant formation of an extra-myofibrillar water pool reflected in a slower-relaxing T_2 population (*3*−*5*). Moreover, combined low-field NMR T_2 measurements and simultaneous impedance measurements, the latter being considered an indirect measure of membrane integrity (*6*), have indicated that the postmortem transmission of myocellular water into extra-fibrillar spaces proceeds simultaneously with disruption of the membrane integrity (*4*). Accordingly, membrane properties seem to play a key role in the postmortem cascade of reactions, in agreement with the fact that the plasma membrane in muscle cells is highly organized and involved in maintaining cellular homeostasis to a much higher extent than previously expected (*7*). Techniques for studying membrane properties in intact muscles are limited. Nonetheless, recently solid-state ^{13}C cross-polarization (CP) magic-angle spinning (MAS) nuclear magnetic resonance (NMR) spectroscopy performed on frozen porcine muscle biopsies, taken during the conversion of muscle to meat (1 min, 45 min, and 24 h postmortem), has indicated that this method has potential, since the data provide information regarding both

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membrane properties and glycogen content in the muscle (*8*). Consequently, a combination of ^{13}C CP MAS NMR spectroscopy and NMR T_2 relaxation time measurements should enable an evaluation of the relationship between characteristics of the muscle membranes and the water distribution in muscles. Hypothesizing that muscle membrane destabilization and disintegration, which potentially is reflected in ^{13}C CP MAS NMR spectra, is involved in the postmortem mobilization of water pools in muscles, which can be measured using NMR T_2 relaxometry, we performed low-field NMR T_2 measurements continuously over a 23-h period on porcine muscle samples and low-temperature ^{13}C CP MAS NMR spectroscopy experiments on equivalent frozen muscle biopsies. Accordingly, the aim of the present study is to evaluate the potential of combining the information obtained from the two techniques.

MATERIALS AND METHODS

Animals and Sampling. The experiments included eight slaughter pigs, which were offspring of Duroc/Landrace boars cross-bred with Landrace/Yorkshire sows and had a live weight of approximately 100 kg. Before the experiments were initiated, the pigs were allocated to four different treatments in a 2 × 2 complete balanced block design where the two factors were (i) preslaughter exercise which consisted of exercise on a treadmill at a speed of 3.8 km/h for 20 min (*9*) versus no preslaughter exercise and (ii) CO_2 stunning (immersion in 80% CO_2 for 3 min using a commercial facility) versus electrical stunning, which was done by placing stunning tongs on the pig's head (220V, 15 s, equipment from K. Schermer & Co., Ettlingen, Germany).

The day before slaughter, a control muscle biopsy sample was taken at the last rib curvature in the right *M. longissimus dorsi* from each pig. Immediately after sampling, the biopsies were frozen in 7 mm o.d. zirconia rotors (225 μL sample volume) (used for the ^{13}C CP MAS NMR spectroscopy) in liquid nitrogen and stored at −80 °C until further analysis. The pigs were slaughtered in the experimental abattoir at Research Centre Foulum. Immediately after exsanguination, a sample of approximately 5 cm was taken from each pig of *M. longissimus dorsi* 3 cm from the biopsy sampling in the cranial direction. From this sample, a subsample (approximately 3.5 cm long and 1 × 1 cm^2 in cross-sectional area) was cut along the fibers using a scalpel. The sample was immediately placed in a cylindrical glass tube with the fiber direction perpendicular to the tube wall and inserted in the low-field NMR analyzer for continuous T_2 relaxation time measurements (see below). At 45 min postmortem, muscle biopsy samples were taken in *M. longissimus dorsi* and instantly frozen in 7 mm o.d. zirconia rotors (used for the ^{13}C CP MAS NMR spectroscopy) using liquid nitrogen and stored at −80 °C until further analysis (see below).

pH and WHC Measurements on the Carcasses. pH and temperature were measured 1 and 45 min postmortem. pH was measured with an insertion electrode (Metrohm AG CH-9101 Herisau, Switzerland) equipped with an glass electrode (Hamilton Tiptrode P/N: 238′080, Switzerland) in the right *M. longissimus dorsi* at the last rib curvature next to the biopsy sampling. The electrode was calibrated at a temperature of 35 °C, and a two-point calibration was performed with calibration buffers of 7.000 and 4.005 at 25 °C (Radiometer, Denmark). Temperature was measured with a Testo 110 thermometer (Testo, Germany).

Measurement of WHC was performed using a modified version of the Honikel bag method (*10*), which is a gravimetric method. A chop for determination of water-holding capacity (WHC) was cut at 24 h postmortem below the sample taken for the low-field NMR measurements. The meat chop with a weight of approximately 80−100 g was trimmed and weighed 24 h postmortem. Subsequently, the sample was placed in a net and then hung in an inflated plastic bag for 24 h at 4 °C, after which it was weighed again. The drip loss was calculated as the difference in weight before and after hanging.

Low-Field NMR Measurements. The relaxation measurements were performed on a Maran Benchtop Pulsed NMR Analyzer (Resonance Instruments, Witney, UK) with a magnetic field strength of 0.47 T and a corresponding resonance frequency for protons of 23.2 MHz. From 10 min postmortem and until 23 h postmortem, transverse relaxation times (T_2) were measured continuously every 10 min using the Carr−Purcell−Meiboom−Gill (CPMG) sequence (*11*, *12*). The T_2 measurements were performed with a τ-value (time between the 90° pulse and 180° pulse) of 150 μs and using a relaxation delay of 3 s, and data were acquired as the amplitude of every second echo (to avoid influence of imperfect pulse settings) in a train of 4096 echoes (only the even numbered echoes were used in the data analysis). A total of 16 scans were accumulated.

The Maran benchtop NMR instrument was equipped with an 18 mm temperature variable probe. By NMR instrument programming (in-house-made script), it was possible to have the temperature regulated automatically during the measuring period, and the temperature of the instrument was set equal to the temperature decrease that the carcass experienced during the slaughtering and cooling process at the experimental abattoir.

Distributed exponential fittings of CPMG decay curves (*13*) were performed in Matlab (The Mathworks Inc., Natick, MA) using in-house scripts. The data were pruned from 2048 to 256 points using linear pruning, which on synthetic data was found to give robust solutions. This analysis resulted in a plot of relaxation amplitude for individual relaxation processes versus relaxation time. From such analyses, time constants for each process were calculated from the peak position, and the area under each peak (corresponding to the proportion of water molecules exhibiting that relaxation time) was determined by cumulative integration. Additionally, the width of the relaxation population was calculated as the standard deviation of the observed relaxation times for the given peak.

^{13}C CP MAS NMR Experiments. ^{13}C CP MAS spectra were recorded on a Varian Unity INOVA-300 (7.05 T) spectrometer at 75.43 MHz. All experiments were performed with a 7-mm home-built high-speed spinning variable-temperature ^{1}H−X double resonance probe tuned to 299.1 and 75.4 MHz. The measurements on the stored (−80 °C) sample rotors were performed at a temperature of −50 °C at the inlet to the probe. This corresponds to an actual sample temperature of −38 °C, determined in separate experiments as described elsewhere using ^{207}Pb MAS NMR of $Pb(NO_3)_2$ as an NMR thermometer (*13*). The rotors were rapidly (in less than 15 s) transferred to the precooled stator in order to avoid deterioration of the samples. A spinning speed of 4.5 kHz was used along with radio frequency (rf) field strengths of approximately 40 kHz for the Hartmann−Hahn match and approximately 65 kHz for ^{1}H decoupling. A contact time of 1 ms and a repetition delay of 4 s were employed. The contact time of 1 ms was chosen because it results in close to optimum spectral intensities for carbons with weak dipolar couplings to protons (e.g., carbonyls), as well as for carbons with strong dipolar couplings to protons. Between 1200 and 1800 scans were accumulated for each sample.

^{13}C chemical shifts are reported relative to TMS, and the scale was referenced using hexamethylbenzene as a secondary substitution standard.

Biochemical Determination of Glycogen and Lactate. After ^{13}C CP MAS NMR spectroscopy, the concentration of muscle glycogen was determined in the samples in duplicate for 50 mg of muscle tissue. Samples were heated in a test tube with 5 mL of 1 M HCl at 100 °C for 2 h to hydrolyze the glycogen to glucose units and then centrifuged at 1500 g for 10 min at 4 °C. The concentration of muscle lactate was determined in duplicate in 10-mg muscle samples, incubated 30 min on ice in vials containing 600 μL of 3 M perchloric acid. The extraction procedure was stopped by adding 1000 μL of 2 M $KHCO_3$ to the vials, and the samples were centrifuged using the same procedure as described for glycogen. Both glucose and lactate were measured spectrophotometrically as outlined elsewhere (*15*) using a Cobas ABX Mira Plus autoanalyzer (ABX Diagnostics, France). The results are expressed in micromoles of glucose residues per gram of muscle (wet weight).

Data Analyses. Statistical analysis was carried out with the Statistical Analysis System (SAS Institute, 1991). Linear regressions were performed using PROC REG, and analysis of variance was performed using PROC GLM. The statistical models in analysis of variance included the fixed effects of preslaughter exercise and stunning method.

In addition, partial least-squares regressions (PLSR) (*16*) were carried out with the Unscrambler version 8.0 software (Camo AS, Oslo,

Table 1. Least-Squares Mean Values and Standard Error (SE) for pH, Temperature, and Drip Loss[a]

	CO_2 stunning/÷exercise ($n = 2$)	CO_2 stunning/+exercise ($n = 2$)	electrical stunning/÷exercise ($n = 2$)	electrical stunning/+exercise ($n = 2$)
pH 1 min pm	6.82 (0.06)a	6.71 (0.06)a	6.46 (0.06)b	6.06 (0.06)c
pH 45 min pm	6.58 (0.10)a	6.58 (0.10)a	6.25 (0.10)a	5.44 (0.10)b
temperature 1 min pm	39.9 (0.4)a	39.9 (0.4)a	40.8 (0.4)ab	41.4 (0.4)b
temperature 45 min pm	40.7 (0.3)ab	39.9 (0.4)a	41.4 (0.4)ab	41.5 (0.4)b
drip loss (%)	1.7 (1.0)a	2.1 (1.0)a	10.9 (1.0)b	11.0 (1.0)b

[a] Different letters (a,b,c,d) in rows indicate significant differences ($P < 0.05$). pm: postmortem.

Norway). PLSR is a multivariate calibration method, by which two sets of data, *X* (e.g., NMR data) and *y* (e.g., glycogen content) are related by means of regression. The purpose of PLSR is to establish a linear model, which enables the prediction of *y* from the measured spectrum *X*. This is described by eq 1:

$$y = X\mathbf{b} \quad (1)$$

in which vector **b** contains the regression coefficients determined during the calibration procedure. PLSR seeks the systematic part of the matrix *X* that is relevant for the description of *y*, and analysis of *X* and the model between *X* and *y* are performed simultaneously. T2 relaxation curves and ^{13}CP MAS NMR spectra were included as *X*-data sets, and glycogen content, lactate content, and T_{22} population size were included as *y*-variables.

During regression, Martens' uncertainty test (*17*) was used to eliminate noisy variables, and all models were validated using full cross-validation ("leave one out"), and only validated results are reported. As indicator of prediction ability, the root mean square error of cross-validation (RMSECV) is given. RMSECV is calculated according to eq 2:

$$\mathrm{RMSECV} = \sqrt{\frac{\sum_{i=1}^{n}(\hat{y}_i - y_i)^2}{n}} \quad (2)$$

in which $\hat{y}_i$ is the predicted value of the *y*-variable for sample *i*, y_i is the actual value of the *y*-variable for sample *i*, and *n* is the number of samples.

RESULTS

pH, Temperature, and WHC Measurements. pH and temperature in the muscles at 1 and 45 min postmortem together with the WHC data are given in **Table 1**. A combination of lower pH and higher temperature are observed in muscles from electrically stunned pigs, which results in higher drip loss compared with muscles from CO_2-stunned pigs, and the effect is further enhanced upon exercise. However, no significant effects of exercise on pH, temperature and WHC are obtained in muscles from CO_2-stunned pigs.

Low-Field NMR T_2 Relaxation Measurements. Figure 1 displays a typical example of CPMG data obtained upon distributed exponential fitting. In all muscles, three components, hereafter referred to as T_{2A}, T_{2B}, and T_{2C}, are detected: a minor component between 1 and 10 ms (T_{2A}), a major component between 30 and 50 ms (T_{2B}), and finally a third component between 150 and 500 ms (T_{2C}). **Figure 2A** and **B** show observed changes in the T_{2B} time constant and the T_{2C} population during the postmortem period. Initially, an increase in the T_{2B} time constant is observed; thereafter it decreases slightly and finally becomes constant (**Figure 2A**). From 10 min postmortem and until about 400 min postmortem, the T_{2C} population is characterized by an increase whereupon it almost stabilizes.

^{13}C CP MAS NMR Measurements. Figure 3 illustrates a typical ^{13}C CP MAS spectrum obtained for a muscle biopsy taken 45 min postmortem. Several resonance lines are detected, which have tentatively been identified and are presented in the figure caption. From the entire ^{13}C CP MAS spectrum, the prediction of glycogen and lactate was investigated. **Table 2** displays the results from PLS regressions, and **Figure 4A** and **B** show the significant variables in the regression analysis. The

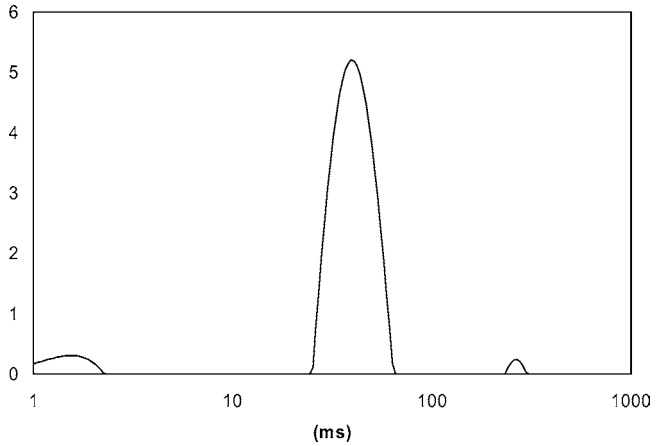

Figure 1. Distribution of the water T_2 relaxation times in a muscle sample measured 50 min postmortem.

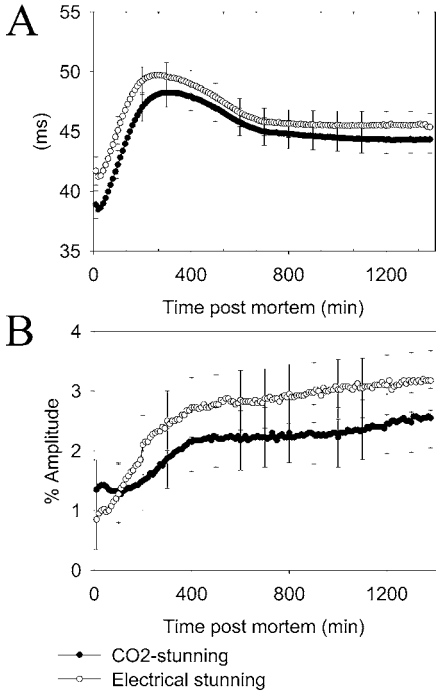

Figure 2. Postmortem course of the T_{2B} time constant (A) and the relative population of the T_{2C} component (B) in muscle samples from CO_2-stunned ($n = 4$) and electrically stunned ($n = 4$) animals. Least squares means are given. Bars indicate standard errors.

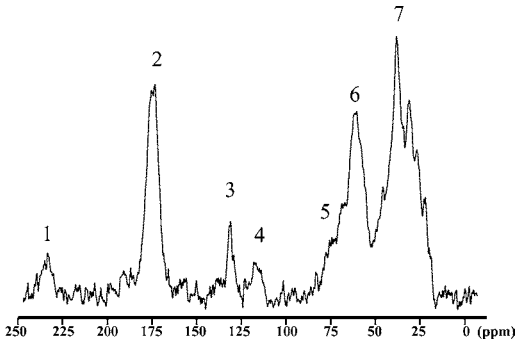

Figure 3. Example of ^{13}C CP MAS spectrum obtained on a frozen muscle sample taken 45 min postmortem (same animal as that in **Figure 1**). The main resonances observed are assigned as follows: (1) (∼235 ppm) + (4) (∼115 ppm), spinning sidebands; (2) (∼175 ppm), carbonyl carbons; (3) (∼130 ppm), unsaturated carbons of fatty acids; (5) (∼72 ppm), C3, C4, C5, and C6 in glycogen; (6) (∼50–58 ppm), methyl groups of choline and phosphatidylcholine; (7) (∼30–35 ppm), various saturated carbons of methylene chains of the fatty acids.

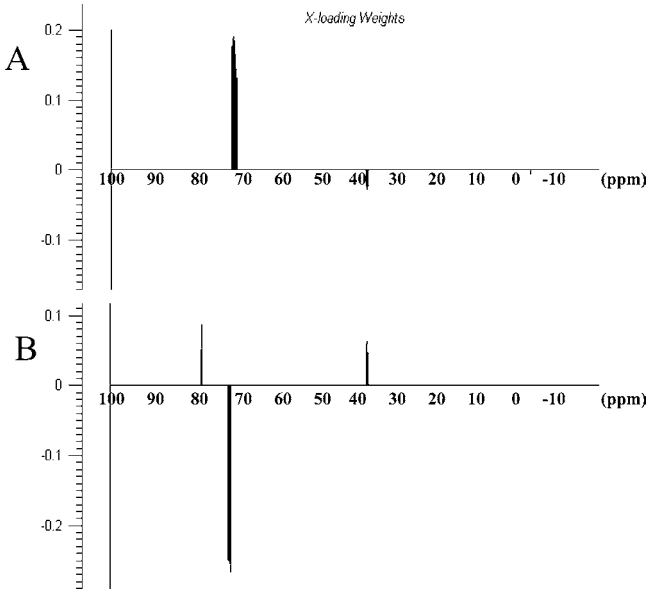

Figure 4. Loading weighting plot (PC1 + PC2). This plot allows elucidation of the resonances that are key in partial least-squares regressions with ^{13}C CP MAS spectra as *X*-variable and with (A) glycogen and (B) lactate as *y*-variable, respectively.

Table 2. Performances of PLS Regression Models with ^{13}C CP MAS Spectra Measured on Muscle Biopsies Taken 45 min Postmortem ($n = 8$) as *X*-Variables and Lactate/Glycogen Content Determined Biochemically as *y*-Variable

variable in *y*	*R*	*y*-var (%)	RMSECV (%)	PC[a]
glycogen	0.89	89	8.4	2
lactate	0.70	70	24.0	2

[a] The number of PCs resulting in the lowest RMSECV were chosen.

correlation between the ^{13}C CP MAS spectra and the glycogen content determined biochemically is high ($r = 0.89$). Illustration of loading weights in the PLS regression shows that the correlation between the ^{13}C CP MAS spectra and the glycogen content can be ascribed to an area in the ^{13}C CP MAS spectra around 72 to 75 ppm (positive correlation) and to an area around 37.5 to 38.5 ppm (negative correlation). Also the correlation between the ^{13}C CP MAS spectra and the lactate content is

Table 3. Performances of PLS Regression Models with ^{13}C CP MAS Spectra Measured on Muscle Biopsies Taken 45 min Postmortem ($n = 8$) as *X*-Variables and T_2 Variables Measured at 50 min Postmortem and Drip Loss Measured 24 h Postmortem as *y*-Variable

variable in *y*	*R*	*y*-var (%)	RMSECV (%)	PC[a]
T_{2B} time constant	0.21	4	2.8	2
T_{2C} population	0.54	66	0.4	2

[a] The number of PCs resulting in the lowest RMSECV were chosen.

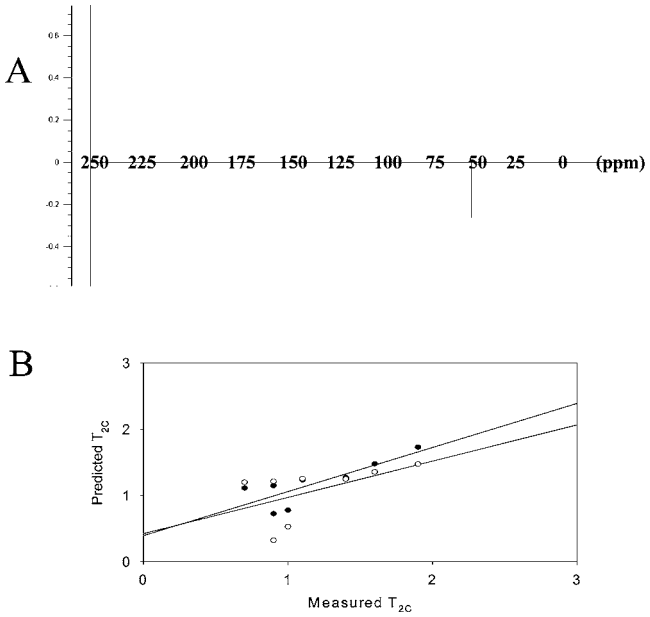

Figure 5. Loading weighting plot. This plot allows elucidation of the resonances that are key in partial least-squares regressions with (A) ^{13}C CP MAS spectra as *X*-variable and the T_{2C} population as *y*-variable and (B) plot of predicted (from the ^{13}C CP MAS spectra) versus measured T_{2C} population. Closed symbols: measured values. Open symbols: predicted values.

significant ($r = 0.70$), and this can be ascribed to regions in the ^{13}C CP MAS spectra around 73 to 74 ppm (negative correlation) as well as regions around 38 to 37.5 and 81 ppm (positive correlations). In addition, the relationship between the ^{13}C CP MAS spectra and the T_2 relaxation data has been examined with the purpose of testing the ability of the ^{13}C CP MAS spectra to predict the various T_2 variables and the drip loss, which is known to correlate with T_2 variables; see **Table 3**. The ^{13}C CP MAS spectra on muscle biopsies taken 45 min postmortem explains 33% of the variation in the T_{2C} population measured at 50 min postmortem, which can be ascribed entirely to the 52 ppm signal intensity in the ^{13}C CP MAS spectra (**Figure 5A**). In contrast, no correlations are observed between the ^{13}C CP MAS spectra from muscle biopsies taken in vivo and T_2 variables at 50 min postmortem or the drip loss (data not shown).

DISCUSSION

Several preslaughter factors are known to affect meat quality, among these preslaughter stress and stunning method (18−20). In the present study, two levels of preslaughter stress (no stress vs exercise on treadmill) and two stunning methods (CO_2 vs electrical stunning) have been investigated. Even though very few animals are included in the study, significant effects of stunning method on pH, temperature, and drip loss are observed as pH is lower and temperature and drip loss are higher in

postmortem muscles from electrically stunned pigs than in postmortem muscles from CO_2-stunned pigs (**Table 1**). This is agreement with previous findings (*18*, *20*). In contrast, only minor effects of preslaughter exercise on pH, temperature, and drip loss are observed (**Table 1**). Exercise could be expected to increase muscle temperature; however, a tendency for this is observed only in muscles from electrically stunned pigs. In contrast, the effect of preslaughter exercise on pH is more clear, implying that glycolysis to some extent becomes accelerated upon exercise. Due to the limited number of animals in the present study, further studies are needed to confirm this observation. However, the findings are in agreement with a previous study, which likewise observes no effect of preslaughter exercise on muscle temperature postmortem but an effect on pH at 1 min postmortem (*9*).

Several reports on the use of T_2 relaxation time measurement to study water distribution in postmortem muscles exist (*3*−*5*, *21*). These have shown an initial increase in the T_{2B} time constant postmortem together with an elongated ongoing increase in the T_{2C} population (*5*), as also observed in the present study. The increase in T_{2C} population is believed to reflect formation and compartmentalization of extra-myofibrillar water (*5*), which is susceptible to being lost as drip (*22*, *23*). It still remains unexplained which physical processes in the muscles initiate these changes in the relaxation characteristics of water. However, a study involving low-field NMR T_2 measurements on porcine muscles and simultaneous measurements of impedance indicates that the increase in the T_{2C} population is associated with the onset of membrane disintegration (*4*). In a recent ^{13}C CP MAS study, it is suggested that ^{13}C CP MAS spectra obtained on frozen muscle biopsies contain information about membrane integrity within the muscle as variations in the signal intensity of a resonance line at approximately 34 ppm are observed between samples, expected to reflect differences in membrane properties (*8*). In the present study, this finding has been investigated further as ^{13}C CP MAS spectra are acquired on muscle biopsies where low-field NMR T_2 relaxation times were measured simultaneously. The purpose of acquiring these data is to examine if any relationship exists between these two types of measurements. Partial least squares regression on the entire ^{13}C CP MAS spectra acquired 45 min postmortem reveals that even though the ^{13}C CP MAS spectra only explains 33% of the variation in the T_{2C} population measured at 50 min postmortem, a correlation to the T_{2C} population exists. Loading weights from the analyses reveal that the correlations can mainly be ascribed to the signal intensity of a resonance line at 52 ppm. Methyl groups of choline and phosphatidyl choline are reported to resonate around 50−55 ppm (*24*), and accordingly the present data suggest that the size of the T_{2C} population is related to the membrane compounds choline and phosphatidyl choline, i.e., membrane integrity as suggested by Bertram et al. (*4*). This finding supports the assumption that disintegration and disruption of membrane functions are the basic mechanisms behind the onset of water exudation from muscle cells (*25*).

Muscle glycogen and lactate are crucial in anaerobic glycolysis and pH development postmortem, and they are primary determinants of meat quality. Development of methods that simultaneously assess muscle glycogen and other significant parameters is essential when the basic understanding of preslaughter requisites and meat quality development is investigated. The strong correlation ($r = 0.89$) between solid-state ^{13}C CP MAS spectra and biochemical determinations of muscle glycogen demonstrate the potential of ^{13}C CP MAS in such studies. The strong correlation found in the present study that is superior to a correlation of $r = 0.74$ is obtained in a previous investigation (*8*) and underlines quantitative advantages of multivariate regression on the entire spectrum instead of performing spectral simulations and deconvolution. This is also supported by the correlation between solid-state ^{13}C CP MAS spectra and lactate content in the samples ($r = 0.70$) observed in the present study compared with a previous study, where no correlation between lactate content has been established from the solid-state ^{13}C CP MAS spectra using simple spectral simulations and deconvolution (*8*). This finding is reasonable considering the fact that several peaks overlap with the methyl group resonance of lactate at ∼21 ppm, normally used to estimate lactate. Noticeably, loading weights in the PLS regression reveal no contribution from the ∼21 ppm resonance to the prediction of lactate content, whereas contributions from the ∼38 ppm, ∼72 ppm, and ∼80 ppm resonances are observed. Carbons (C3, C4, C5) in glucose residues are assigned to the resonance at 72 ppm, and accordingly the negative correlation to the 72 ppm resonance is expected, as glucose is the substrate for lactate formation. In contrast, the correlation between the ∼38 ppm and ∼80 ppm resonances and lactate formation is not as obvious, and it remains unclear to which compound the 80 ppm resonance should be assigned. However, the 38 ppm resonance may be assigned to creatine/phosphocreatine (*26*), and an increased signal intensity of creatine/phosphocreatine may result from dephosphylation of phosphocreatine (to creatine), which is known to take place postmortem. Accordingly, the present data suggest that postmortem formation of lactate and degradation of creatine are related.

CONCLUSION

In conclusion, the present study reveals correlation between ^{13}C CP MAS spectra obtained 45 min postmortem and the amount of the slowest relaxing T_2 water population (0.54) at 50 min postmortem. This correlation is obtained from the resonance at 52 ppm, for which the intensity is known to contain contributions from methyl groups in choline/phosphatidyl choline situated in membrane structures. Thereby the present data support previous suggestions that the onset of an increase in the T_{2C} population is associated with the onset of membrane disintegration. In addition, performing PLS regression on the entire ^{13}C CP MAS spectra, the present study shows that ^{13}C CP MAS spectra explain 89% and 70% of the variation in glycogen and lactate content, respectively. Analysis of loading plots reveals that postmortem changes in lactate are related to changes in the 38 ppm signal intensity. Accordingly, the present data suggest a relationship between postmortem formation of lactate and properties of creatine. Further studies should be carried out to verify this finding.

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