Investigation of temperature dependence of time-of-death estimation based on 1H-MRS measurements in sheep heads

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Introduction: It has been demonstrated that an objective and reliable estimation of the postmortem interval (PMI) can be done using ^{1}H-MRS to characterize brain decomposition [1, 2]. Parameterized functions that describe the time courses of concentration changes of various metabolites at room temperature thereby enable the estimation of the postmortem interval (PMI) [2]. However, biochemical processes and, consequently, metabolite-based determination of PMI strongly depend on ambient temperature. In order to quantify the influence of temperature, the present work investigated sheep brain decomposition at temperatures between 4°C and 26°C. In addition, this work aimed at an extension of the current model, which is restricted to room temperature and where changes of metabolite concentrations are a function of PMI only $y=f(t)$, to a model that includes the temperature $y=f(t,T)$ as well.

Methods: Single-voxel PRESS ^{1}H-MRS (TR=3s, TE=20ms, water and outer volume suppression) was used to investigate sheep brain decomposition *in situ* at different ambient temperatures (4@4±2°, 6@11±1°, 4@19±1° and 4@26±0°C). Spectra were recorded on a 1.5 Tesla whole body scanner (GE SIGNA) using a conventional quadrature head coil. Sheep heads were placed in plastic containers and stored in a conditioning cabinet during the entire period of investigation (except for short periods of the MR examination). Two voxels of 10×10×15mm^3 were measured in the parieto-occipital region of the brain (one in each hemisphere). Spectra were quantified with LC-Model [3] using an extended basis set of metabolites [1] and the fully relaxed water signal as internal concentration standard.

Results: From all 27 components evaluated (for a listing see [1]), 20 metabolites showed reproducible time courses; either for the entire period investigated or for large parts. However, all metabolites are not appropriate for further use in PMI estimation due to ambiguous time courses. A total of seven metabolites (total Creatine (Cr_{tot}), Glycerol (Glol), Myo-Inositol plus Glycine (mI&Gly), Lactate (Lac), Acetate (Ace), free Trimethylamonium (fTMA) and NAA plus NAAG (NA_{tot})) show significant sudden concentration changes at all temperatures investigated. This is illustrated in Figure 1 for Cr_{tot} where the critical time-points are indicated by a dashed red line. Each color/symbol thereby represents a single sheep head and each point the average of two measurements (one from each brain hemisphere). The time-points immediately before the sudden change (moment of last MR-measurement) were evaluated for all seven metabolites and summarized in Figure 2 and an exponential curve was fitted to the data (red line). Before this critical time-point, all 20 metabolites show reproducible time courses, whereas after this moment this remains not true for all metabolites. Therefore upper limits for PMI estimation ($t_{max.}$) corresponding to the critical time-point were calculated for each temperature based on the fitted curve (Fig 2). For further analysis only data with $t<t_{max}$ were included. For $t<t_{max}$ ten metabolites (Ace, Ala, GABA, Valine (Val), Butyrate (But), Propionate (Prop), Succinate (Suc), Glol, Phospho-Ethanolamine (PE) and mI&Gly) showed a linear increase and NA_{tot} a linear decrease (Glol and NA_{tot} after a log transformation). A linear regression curve $f(T)=m\cdot T+b$ was fitted to the data (an example is shown in Figure 3) of all eleven metabolites and temperature dependency of slope (m) and y-axis intercept (b) were evaluated. Seven metabolites show an unambiguous relation of slope (m) and temperature that resembles an exponential increase (Figure 4). NA_{tot} shows a linear decrease and only for Prop, PE and Glol the slope (m) does not depend on temperature (linear regression analysis reveals $p>0.3$).

Figure 1: Concentration of total Creatine

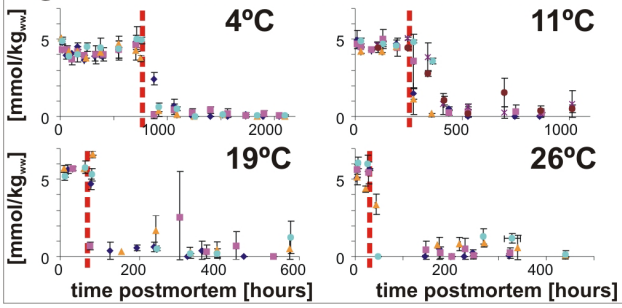


Figure 2: Start of bacterial decomposition

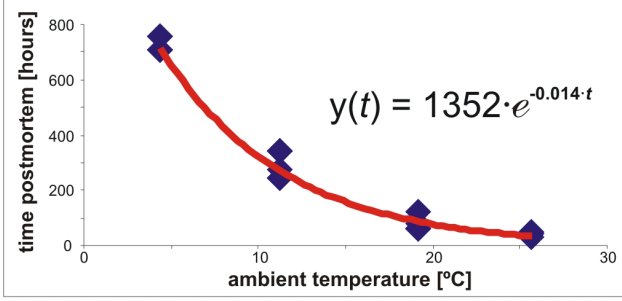


Figure 3: Alanine concentration

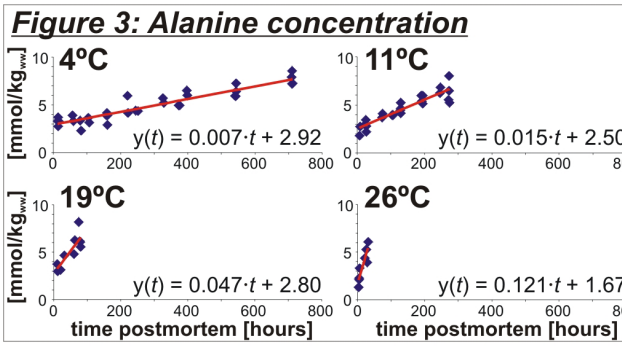
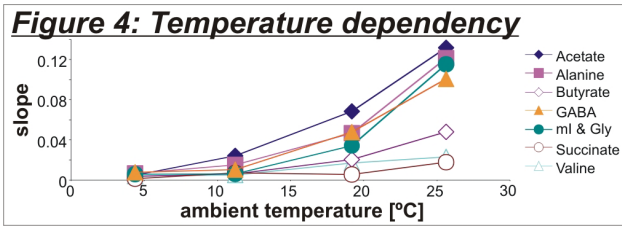


Figure 4: Temperature dependency



Discussion & Conclusions: The sudden change of the concentrations (Figure 1) of seven metabolites and the clustering of the corresponding time-points (Figure 2) in combination with a drastic increase (data not shown) of fTMA, But, Prop and Suc – metabolites indicative of bacterial metabolism – allow the conclusion that the critical time-points (t_{max}) indicate the onset of bacterial decomposition. Thereafter some of the metabolites show either ambiguous or even un-reproducible time courses (data not shown). Therefore data analysis was restricted to data with $t<t_{max}$. Before the onset of bacterial metabolism eleven metabolites showed unambiguous concentration changes that could be fitted to linear functions. The slope of the fitted curves can be interpreted as a measure for the activity of the biochemical reaction that leads to the metabolite concentration changes. As expected, these values show an increase with increasing temperature for seven of the metabolites; even showing similar (presumably exponential) behavior (see Figure 4). Thus, for these seven metabolites and for NA_{tot} the individual time courses can be described with single functions that include PMI and temperature $y=f(t,T)$. Therefore, a combined application of the inverse functions $t=f'(y,T)$ can be used for PMI estimation [2] at different ambient temperatures between 4°C and 26°C until the onset of bacterial decomposition.

References: **[1]** Ith M et al. Magn Reson Med 2002;**48**:915-920; **[2]** Scheurer E et al. NMR Biomed 2005;**18**:163-172; **[3]** Provencher SW et al. Magn Reson Med 1993;**30**:672-679