BIOPHYSICS AND BASIC BIOMEDICAL RESEARCH – Rapid Communication

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Acute Porcine Renal Metabolic Effect of Endogastric Soft Drink Administration Assessed with Hyperpolarized [1-13C]pyruvate

Christoffer Laustsen,[1]* Esben Søvsø Szocska Hansen,[1,2] Uffe Kjærgaard,[1] Lotte Bonde Bertelsen,[1] Steffen Ringgaard,[1] and Hans Stødkilde-Jørgensen[1]

Purpose: Our aim was to determine the quantitative reproducibility of metabolic breakdown products in the kidney following intravenous injection of hyperpolarized [1-^{13}C]pyruvate and secondly to investigate the metabolic effect on the pyruvate metabolism of oral sucrose load using dissolution dynamic nuclear polarization. By this technique, metabolic alterations in several different metabolic related diseases and their metabolic treatment responses can be accessed.

Methods: In four healthy pigs the lactate-to-pyruvate, alanine-to-pyruvate and bicarbonate-to-pyruvate ratio was measured following administration of regular cola and consecutive injections of hyperpolarized [1-^{13}C]pyruvate four times within an hour.

Results: The overall lactate-to-pyruvate metabolic profile changed significantly over one hour following an acute sucrose load leading to a significant rise in blood glucose.

Conclusion: The reproducibility of hyperpolarized magnetic resonance spectroscopy in the healthy pig kidney demonstrated a repeatability of more than 94% for all metabolites and, furthermore, that the pyruvate to lactate conversion and the blood glucose level is elevated following endogastric sucrose administration. **Magn Reson Med 74:558–563, 2015.**

Key words: MRI; MRS; sucrose; kidney; renal metabolism; hyperpolarization

INTRODUCTION

Dissolution dynamic nuclear polarization (d-DNP) is a novel MR imaging and spectroscopic technique that has demonstrated the ability to quantify metabolic rates after injection of endogenous agents in situ (1). These agents are labeled with the nonradioactive stable isotope ^{13}C in a specific molecular position and are hyperpolarized with a dedicated laboratory equipment to create a magnetization (more than 10,000 enhanced signal) detectable by a MR system (2). The use of hyperpolarized contrast agents provides an opportunity to combine the flexibility and safety of MR-based imaging with an exceptional signal-to-noise ratio. Exploration of injectable ^{13}C-labeled substances has only recently entered clinical trials in prostate cancer patients (3). Hyperpolarized ^{13}C-labeled pyruvate has recently been proposed as a possible endogenous marker to identify renal metabolic changes in diabetes patients (4–7). However, to translate the promising findings in rodent models to humans, large animal models play a vital role as their metabolic profile, response and organs size yield a more realistic model for the human reproducibility of the technique.

The metabolic alterations following endogastric sucrose load was investigated to determine the metabolic dependence on feeding status in the porcine kidney, as previous studies indicate that fructose (sucrose derivative) administration in healthy controls upregulates renal lactate exchange (8). A soft drink Coca Cola regular (Denmark, sucrose sweetened) contains sucrose (table sugar) as the sweetener; however, it is important to note that there are regional differences on Coca Cola composition, with high corn fructose syrup (HCF) being the sweetener in the United States.

Currently no noninvasive methods are able to assess abnormal response to oral glucose administration in patients with a creatinine clearance above 86.5 mL min^{-1} m^{-2} (9), illustrating the essential requirements for new methods for assessing the early changes in the diseased kidney, we here introduce hyperpolarized MRS as a novel method to this end.

METHODS

Healthy 30 kg female Danish landrace pigs (N = 4) were included in this study. All animals were treated according to the Danish law on animal experiments (J.nr.: 2014-15-2934-01013). The pigs were sedated with an intramuscular injection containing a mixture of Stressnil

[1]MR Research Centre, Aarhus University Hospital, Aarhus, Denmark.

[2]Danish Diabetes Academy, Odense, Denmark

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*Correspondence to: Christoffer Laustsen, Ph.D., MR Research Centre, Aarhus University Hospital, Palle Juul-Jensens Boulevard 99, DK-8200 Aarhus N, Denmark. E-mail: cl@clin.au.dk

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(2.0 mg/kg bodyweight) and Midazolam (0.1 mL/kg). Intravenous (i.v.) access was obtained through an ear venflon. Each pig received 12 mg propofol (B. Braun Medical A/S) i.v. and were intubated. Anesthesia was maintained with propofol (0.4 mg/kg/h). The pigs received i.v. fentanyl (8 μg/kg/h) for pain relief. The pigs were mechanically ventilated (60% oxygen) with a respirator. The respiratory rate was adapted during the experiment to keep expiratory CO_2 rate at a level between 4.5 and 5.5% to minimize respiratory acidosis and respiratory frequency was increased from ~12/min pre to ~16/min post to obtain this. Images were acquired during temporary suspension of the ventilator to avoid motion artifacts. A bladder catheter was inserted. Guided by ultrasound a 6F catheter was inserted into the femoral artery to obtain arterial blood samples. Additionally, a 6F femoral vein catheter was applied for administration of hyperpolarized [1-^{13}C]pyruvate. The catheters were both flushed with heparin (20 IU) dissolved in 10 mL of isotone sodium chloride. Finally, a gastric tube for administration of the fluid sugar load mixture was applied.

Two baseline fasted d-DNP metabolic images was acquired with 20 min separation to identify the reproducibility in the fasted state. Hereafter a degassed sucrose-sweetened soft drink 660 mL (regular Coca Cola, DK), with 10.6 g sucrose per 100 mL leading to a total amount of 70 g (2.33 g/kg) sucrose was administered by means of the gastric tube in approximately 2 min, yielding by means of full hydrolysis approximately 33 g glucose and 33 g fructose. To mimic a realistic meal electrolyte mixture containing a total of 84 mg magnesium, 105 mg potassium, 13.5 mg calcium, 42 mg Vitamin C, 300 mg sodium, and 1.5 mg green tea extract (ZERO, HIGH5, UK) was dissolved in the Coca Cola and coadministrated. After a wait of 20 and 40 min d-DNP hyperpolarized ^{13}C metabolic examinations was performed to identify the metabolic effect of the nonfasted state (Fig. 1). Every 10 min, the blood glucose was measured in arterial blood with a Contour XT blood glucose meter (Bayer Health Care, Copenhagen, Denmark). Before the first and after the last imaging experiment arterial blood samples were taken from the catheter and were analyzed with an ABL 700 Series Blood Gas Analyzer (Radiometer, Copenhagen, Denmark). After the experiment the pigs were euthanized by i.v. Pentobarbital (100 mg/kg). A single control animal underwent the full procedure with arterial blood samples taken every 10 min, without soft drink administration to investigate the blood gas status over 60 min, with repeated injections of hyperpolarized pyruvate to identify effects of prolonged anesthesia, repeated injections of high concentration pyruvate and breath holding.

Sample Preparation

Four identical samples of 600 mg of [1-^{13}C]pyruvic acid mixed with 15mM AH111501 (trisodium salt of tris{8-carboxyl-2,2,6,6-tetra[2-(1-methoxyethyl)]-benzo(1,2-d:4,5-d')bis(1,3)dithiole-4-yl}methyl acid was polarized in a SPINLab (GE Healthcare, Broendby, Denmark) polarizer (10) for more than 2.5 h at 5 Tesla (T) at 0.8 K, to ensure a reproducible polarization of 30% on average. Dissolution was performed with 20 mL of de-ionized water into a receiver syringe, containing 16.3 mL of 360 mM sodium hydroxide (NaOH) and 180 mM tris(hydroxymethyl)aminomethane (TRIS) for neutralization of the pyruvic acid. Approximately 30 mL of 250 mM pyruvate concentration (20 mg/kg), similar to the dose administered in Nelson et al (3), was administered intravenously in 5 s in the femoral vein.

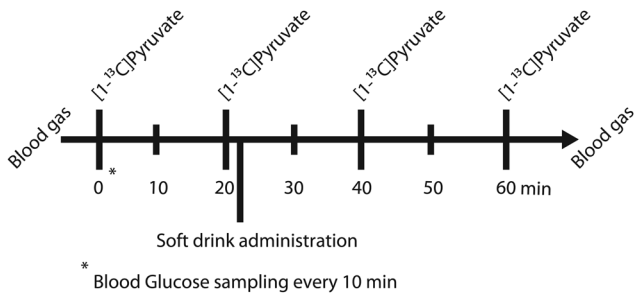

FIG. 1. Study design illustration, endogastric regular Coca Cola administration was performed after the second fasted hyperpolarized examination (20 min). The initial blood gas measurement was followed by blood glucose measurements every 10 min and hyperpolarized [1-^{13}C]pyruvate MRS experiments every 20 min. The experiment is completed with a final blood gas measurement.

MR Spectroscopy Imaging

The MR experiments were performed on a 3T GE Hdx, equipped with a ^{13}C Helmholtz loop coil (PulseTeq Limited, Surrey, UK) (Ø = 20 cm) covering both kidneys. Scout images were performed with the ^{1}H body coil to localize the kidneys and to position the ^{13}C coil. For anatomical ^{1}H imaging a T2-weighted fast spin echo sequence was used in the axial orientation with a repetition time/echo time (TR/TE) 5040 ms/109 ms, flip angle of 90, 30 cm field of view (FOV), 384 × 384 matrix, echo train length 28, number of slices 26 and slice thickness 4mm. A standard 2D ^{13}C FID CSI sequence with sequential k-space ordering (11) (19 s total scan time) TR/TE 75 ms/2.5 ms and constant flip angle of 10° was used to image the hyperpolarized pyruvate and its derivatives with a nominal spatial resolution of 1.25 × 1.25 × 1 cm^3, with 20 cm FOV, 16 × 16 matrix, in an axial 1 cm slab covering both kidney and a spectral resolution 19.5 Hz with 256 point and a spectral width of 5000 Hz, was initiated 20 s after end of injection. The mean transfer time from dissolution-to-end-of-injection was 55 s. The CSI was acquired during temporary suspension of the ventilator to avoid motion artifacts.

Processing

The CSI data were processed in MATLAB (MathWorks, Natick, MA) in an in-house MATLAB program. The spatial dimensions were apodized with a hamming function and zero-filled to a 32 × 32 grid. The spectral dimension was apodized with a 0 shifted sine-bell function and a 15 Hz exponential line broadening. Spectral analysis was performed by integration. The processsed data were then imported to OsiriX (12) for anatomical overlay and region of interested (ROI) analysis. Two ROIs was drawn

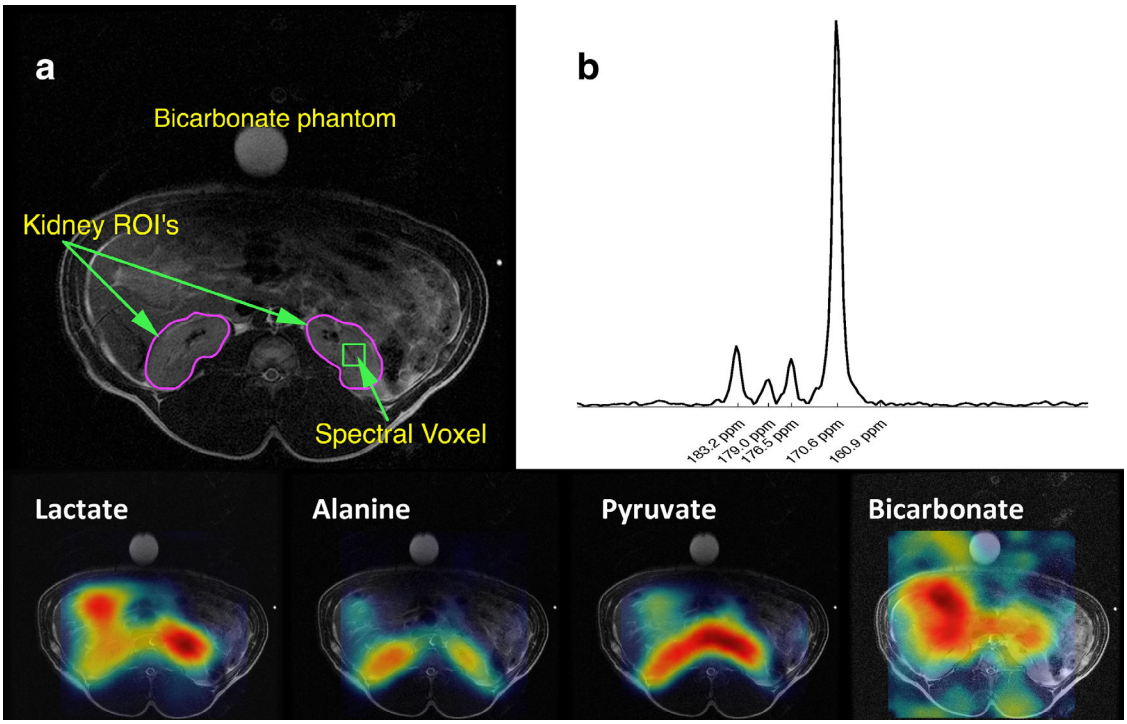

FIG. 2. Illustrative ^{1}H anatomical image with two kidney ROIs and a single voxel outline (1.25 x 1.25 x 1 cm^{3}) (**A**) for the spectrum (**B**). ^{1}H anatomical image overlay showing the individual metabolic maps of ^{13}C lactate (183.2 ppm), alanine (176.5 ppm), pyruvate (170.6 ppm) and bicarbonate (160.9 ppm), showing a high metabolic conversion in the healthy kidneys, images are individually log scaled.

around the kidneys for metabolic profiling (Fig. 2). For each hyperpolarized experiment lactate, alanine and bicarbonate signal was normalized relative to the pyruvate signal, while the pyruvate signal was normalized relative to the total carbon signal.

Statistics

Normality was assessed with quantile-quantile plots. A single outlier time point was excluded from the analysis. $P < 0.05$ (*) were considered statistically significant. A one-way repeated measurement analysis of variance were used to compare the metabolic response as a function of time. Statistical analysis was performed in STATA and the plotting was performed in GraphPad, Prism (GraphPad Software, Inc. La Jolla, CA).

RESULTS

Endogastric administration of sucrose was demonstrated to lower the arterial pH level significantly, as well as to increase pCO_2 and blood lactate levels (Table 1).

The lactate-to-pyruvate ratio (Fig. 3A) showed positive correlation with sucrose infusion ($P < 0.001$). Similarly, the pyruvate-to-total-carbon ratio (Fig. 3C) showed a negative dependence on the endogastric sucrose load ($P < 0.001$). The alanine-to-pyruvate (Fig. 3B) ($P = 0.17$) and bicarbonate-to-pyruvate ratio (Fig. 3D) ($P = 0.45$) was indifferent to the sucrose load showing ratios of 0.07 ± 0.02 and 0.013 ± 0.01, respectively.

This suggests that there is 95% chance that another experiment in a healthy porcine kidney will find alanine-to-pyruvate ratio within the confidence interval of 0.062–0.073 and bicarbonate-to-pyruvate ratio within the confidence interval 0.012–0.014, irrespective of the blood glucose level.

An initial fasted state blood glucose level with 4.75 ± 0.8 mmol/L was found at baseline, while the endogastric administration of regular Coca Cola resulted in a significant increase in blood glucose to 10.75 ± 1.8 mmol/L in 40 min (Fig. 3E), a similar yet less significant tendency was observed in the control animal (Fig. 4A). The CO_2 showed a significant increase following the sucrose administration (Fig. 3F), this trend was not observed in the control animal (Fig. 4D). However a tendency toward increased lactate level was observed in the control animal (Fig. 4B). The reliability was assessed by means of the intraclass correlation by comparing the

Table 1
Summary results from arterial blood gas levels obtained before and after glucose administration. Significant results ($p<0.05$) are marked with *.

	Pre	Difference	Post
pH	7.49±0.10	(p=0.01)	7.38±0.10 *
pO_2 (kPa)	40.5±7.13	(p=0.08)	36.23±9.83
pCO_2 (kPa)	4.87±1.0	(p=0.02)	6.16±1.33 *
Lac (mmol/L)	0.7±0.16	(p=0.01)	2.23±0.38 *
Na (mmol/L)	134.50±1.29	(p=0.06)	133.75±0.96
K (mmol/L)	3.98±0.3	(p=0.10)	3.68±0.17
Ca (mmol/L)	1.34±0.03	(p=0.22)	1.33±0.04
Cl- (mmol/L)	101.50±1.29	(p=0.04)	99.75±1.50 *

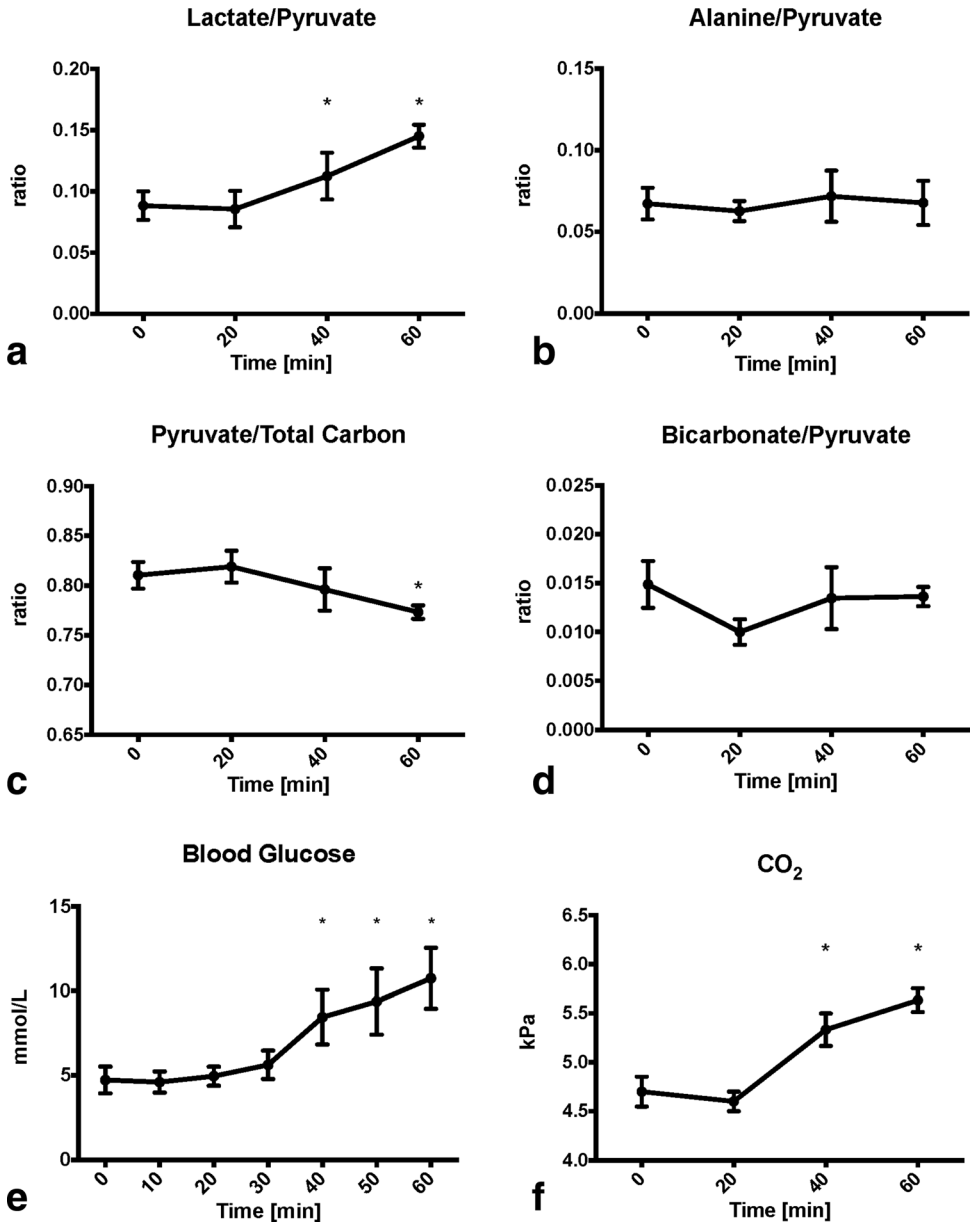

FIG. 3. Lactate-to-pyruvate ratio as function of time (**A**); alanine-to-pyruvate ratio as function of time (**B**); pyruvate-to-total-carbon ratio as function of time (**C**); bicarbonate-to-pyruvate ratio as function of time (**D**); blood glucose as function of time (**E**); CO_2 as function of time (**F**). Significant differences ($P < 0.05$) between the initial control measurements and the results following endogastric soft drink infusion are marked with *. Error bars represent standard error of mean (SEM).

variability within the same subject to the total variation between subjects (Table 2).

DISCUSSION

The novelty of this study was that only the pyruvate-to-lactate conversion was affected by the endogastric administration of 660 mL soft drink, while the pyruvate-to-alanine and pyruvate-to-bicarbonate conversions were largely unaffected. It, however, should be noted that the bicarbonate signal was generally low in the kidney (Fig. 2B) and, therefore, it cannot be ruled out that there might be an effect on the bicarbonate signal. The reproducibility of the examinations, showed great promise as a clinical tool, where the sensitive metabolic fingerprint can aid personalized medicine, with detailed information on the cellular level in real time in a large animal model.

It has previously been shown that deranged metabolism can be found in the diabetic rat kidneys and that this reflects an overstimulation of several pathways (5–7,13) over an extended time period. The acute effect of elevated blood glucose, in absence of diabetes, has though not been associated with alterations in kidney function, albeit significant alterations in the pyruvate metabolism by means of an elevated lactate-to-pyruvate ratio and increased substrate usage in the kidneys following fructose infusion has previously been shown with biochemical analysis (8).

The limitation of the current study is that although pyruvate metabolism is central in glucose metabolism, the metabolic effect of the soft drink containing sucrose and several other confounding factors such as caffeine, oxygenation, anesthesia, fasting status, and repeatable injection of high concentration of pyruvate might lead to alternative metabolic pathways and that the effect

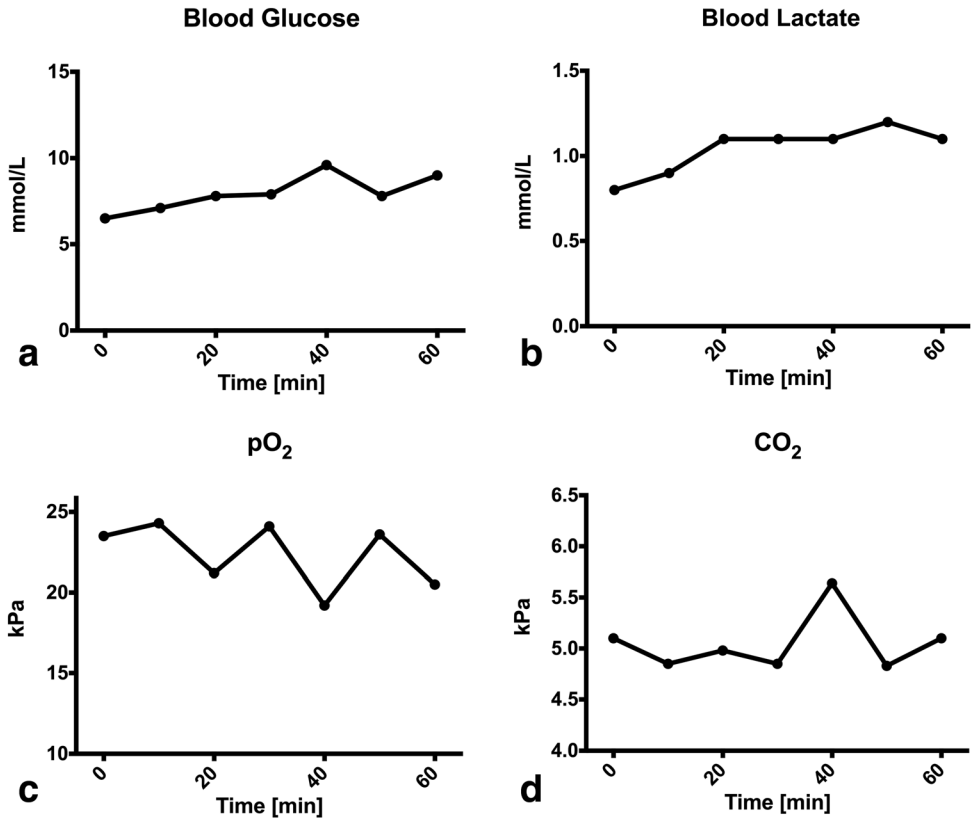

FIG. 4. Blood glucose as function of time (**A**); Blood lactate as function of time (**B**); pO_2 as function of time (**C**); CO_2 as function of time (**D**).

reported here demonstrates the effect of a sucrose sweetened beverage to the fasted kidney leading to both higher blood glucose level and increased lactate-to-pyruvate ratio following the sucrose load. Furthermore, these data do not rule out suboptimal glucose homeostasis, as the blood glucose was not sampled until normal fasting glucose was reached, however, in general the glucose homeostasis is found to be normal in this type of pig. A mechanistic explanation for the observed effects of administration of a sucrose load to the fasted porcine kidney might be explained by known stimulatory effect of insulin, secreted in response to the glucose load. It has recently been demonstrated that insufficient insulin treatment to reverse diabetes increases the lactate-to-pyruvate ratio (7). This support the slow response observed, as the ingestion of sugar and hydrolysis has to take place before the insulin response increases the lactate-to-pyruvate ratio and therefore the response is delayed compared with intravenous glucose administration. This might originate as delayed response to the pyruvate injections, leading to increased blood glucose following gluconeogenesis in the liver and kidney; however, further studies is needed to elucidate this.

The reliability of the current setup demonstrates the potential of hyperpolarized MRS for potential treatment monitoring in patients with renal diseases, where acute metabolic responses can be observed. Imaging of acute metabolic interventions such as glucose infusions increase the specificity compared with current nonimaging methods, representing a novel addition to the current state-of-the-art methods.

Table 2
Within subjects SD in the fasted state, between subjects SD in the fasted state and Intraclass Correlation (ICC) in the fasted state.

	Lactate	Alanine	Pyruvate	Bicarbonate
Within subject SD	0.0048	0.0026	0.0072	0.0005
Between subject SD	0.027	0.011	0.029	0.002
ICC	0.97	0.95	0.94	0.96

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