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Original Article

Hepatic metabolism of ^{11}C-methionine and secretion of ^{11}C-protein measured by PET in pigs

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Abstract: Hepatic amino acid metabolism and protein secretion are essential liver functions that may be altered during metabolic stress, e.g. after surgery. We wished to develop a dynamic liver PET method using the radiolabeled amino acid ^{11}C-methionine to examine this question. Eleven 40-kg pigs were allocated to either laparotomy or pneumoperitoneum. 24 hours after surgery a 70-min dynamic PET scanning of the liver with arterial blood sampling was performed immediately after intravenous injection of ^{11}C-methionine. Time course of arterial plasma ^{11}C-methionine concentration was used as input function and that of liver tissue ^{11}C-concentration as output function in an extended Patlak analysis that accounted for irreversible metabolism of ^{11}C-methionine (hepatic systemic metabolic clearance K_{met}) and secretion of ^{11}C-protein + ^{11}C-metabolites into blood (rate constant k_{loss}). Appearance of ^{11}C-proteins in arterial plasma was measured during the experiment. There were no statistically significant differences between the laparotomy group and the pneumoperitoneum group in any of the calculated parameters. Average mean hepatic systemic metabolic clearance K_{met} was 0.212 mL plasma/mL liver tissue/min, secretion rate constant from liver to blood k_{loss} 0.0054 min^{-1}, flux of methionine F_{flux} 3.59 µmol methionine/mL liver tissue/min, and the appearance rate of ^{11}C-proteins in plasma R_{prot} 0.048 kBq/mL plasma/min. There was significant correlation between K_{met} and R_{prot}. In conclusion, the hepatic systemic metabolic clearance of ^{11}C-methionine was significantly correlated to the appearance rate of ^{11}C-proteins in plasma. It would be interesting to translate the present method to human studies for the development of a clinical quantitative test of hepatic protein secretion.

Keywords: Methionine, liver metabolism, amino acids, hepatic protein secretion

Introduction

Metabolism of amino acids is a vital liver function involving synthesis and secretion of proteins to blood, e.g. albumin and immunoglobulins, but also gluconeogenesis and secretion of acute phase reactants during metabolic stress [1]. Methionine, an essential amino acid, is utilized in the liver for protein synthesis, transmethylation reactions e.g. for phospholipid synthesis, and as precursor for cysteine and taurine [2, 3]. The radiolabeled methionine analog L-[methyl-^{11}C]-methionine (^{11}C-methionine) has been proposed as a tracer for measurement of hepatic protein synthesis in rats and mice, but this is a difficult task because ^{11}C-methionine also undergoes transmethylation for phospholipid synthesis [4-9]. However, the liver is the main contributor to labeled plasma proteins after injection of ^{11}C-methionine in humans [10]. Using positron emission tomography (PET) the metabolism of ^{11}C-methionine was used to measure pancreatic exocrine function in humans [11], salivary gland function after radiotherapy in humans [12], and protein synthesis in muscle tissue in dogs and humans [13, 14].

During metabolic stress, e.g. after surgery, amino acids are released from muscle tissue by protein breakdown and used for catabolic processes in the liver [1]. Laparoscopic surgery provides less postoperative catabolic stress response than laparotomy [15-19]. In the present study we investigated the hepatic metabolism of ^{11}C-methionine by PET and hepatic secretion of ^{11}C-proteins into plasma in pigs subjected to laparotomy compared to pigs sub-

jected to minimal invasive technique by pneumoperitoneum.

Material and methods

Radiotracer production

^{11}C-methionine was produced applying an adopted standard procedure using S-[^{11}C]methylation of homocysteine thiolactone with [^{11}C] methyl iodide in acetone in the presence of 0.3 M sodium hydroxide solution, followed by preparative HPLC [20]. For preparative HPLC a 250 x 10 mm Phenomenex Sphericlone ODS with saline as eluent and a flow rate of 4 mL/min was used. The molar activity, formerly known as specific radioactivity, exceeded 20 GBq/μmol at time of injection for all examinations (tracer release criteria for ^{11}C-methionine).

Animal preparation

Twelve female pigs of Danish landrace weighing 39-42 kg (mean; 41 kg) were included but one pig died during surgery due to hyperthermia and was excluded. The animals were housed and cared for at the animal farm of Aarhus University in accordance with requirements for animal care stipulated by the Danish Animal Experimentations Inspectorate under the Ministry of Justice. Animals were allocated into two groups, laparotomy (n = 6) and pneumoperitoneum (n = 5). Before surgery the animals fasted for 16 hours with free access to water. Anesthesia was initiated by intramuscular injection of ketamine (Ketalar), 10 mg/kg BW and midazolam, 0.5 mg/kg BW, followed by intubation and volume controlled ventilation. *Pneumoperitoneum* was established by insertion of a Veress cannula through a 10-mm subumbilical skin incision and insertion of four trocars, two of 5 mm and two of 10 mm, in the position for laparoscopic cholecystectomy (SurgiPort; United States Surgical Coorporation, Norwalk, CT, USA). Pneumoperitoneum was maintained continuously for 90 minutes. *Laparotomy* was performed by a 15 cm long transverse subcostal incision, mimicking the incision used for an open cholecystectomy. The abdominal wall was opened with electrocautery and a retractor was placed in the wound for 90 minutes and then sutured in two layers. The animals were housed at the animal farm of Aarhus University to the next morning. After completion of the experimental PET procedures described below, euthanasia was performed by an intravenous injection of phenobarbital in a dose of 100 mg/kg. The liver was removed and weighted (mean; 883 g, range; 740-1025 g).

PET examination

24 hours after surgery the animals were anaesthetized, intubated and ventilated for the PET procedure. A catheter was inserted into the femoral vein for injection of ^{11}C-methionine, and another in the femoral artery for arterial blood sampling. The animals were placed in supine position with the liver in the 15-cm field of view of the PET-camera (Siemens ECAT EXACT HR-47 tomograph, CTI/Siemens Medical Systems, Knoxville, USA).

After a 15-minutes transmission scan for attenuation correction, 500 MBq L-[methyl-^{11}C]-methionine, produced in the radiochemistry facility at the PET Centre [20], was injected intravenously over 12 seconds in the beginning of a 70-min dynamic scanning of the liver with time frame structure 12 x 5 s, 3 x 10 s, 3 x 30 s, 7 x 60 s, and 6 x 600 s. Data was reconstructed with filtered back projection using a Hann filter with a cut-off frequency of 0.2, 128 x 128 x 47 matrix and voxel size 2.0 x 2.0 x 3.1 mm^3. Data was recorded as integrated mean values in each of the time frames and corrected for radioactive decay back to start of the scan.

Liver regions of interests (ROIs) were drawn in adjacent slices on the PET image in the last time frame, with a minimum of one cm from the edge in the right liver lobe. ROIs were summed to form a volume of interest (Liver-VOI: mean; 26.2 mL, range; 15.8-34.2 mL) (**Figure 1**).

Blood analysis

Arterial blood samples (1 mL) were taken during the scans at 12 x 5 s, 2 x 10 s, 1 x 20 s, 2 x 30 s, 1 x 45 s, 6 x 60 s, 1 x 330, and 5 x 600 s, a total of 65 minutes (last sample corresponding to mid-point of last time-frame of the PET scan) and plasma ^{11}C-concentrations were measured using a well counter (Cobra II, Packard Instruments Co., Meriden, CT). Fractions of un-metabolized ^{11}C-methionine and of ^{11}C-metabolites in plasma were measured in 2 mL blood samples taken 0, 2, 5, 10, 15, 35, and 55 min after tracer injection. ^{11}C-protein

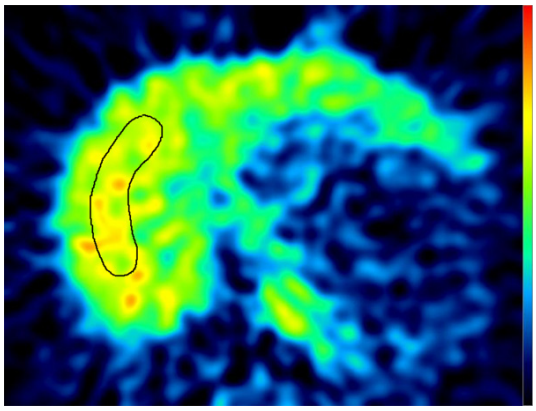

Figure 1. Transaxial slice of the PET image of the liver in the last time frame (Pig 9). Liver-ROI shown in black. Similar adjacent ROIs were summed to form a Liver-VOI.

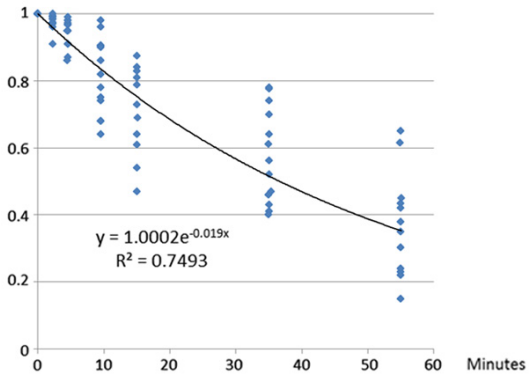

Figure 2. Average time course of the fraction of ^{11}C-methionine concentration in arterial plasma ("free fraction") (y-axis) versus time after injection of ^{11}C-methionine (x-axis; minutes) with all individual data shown along with the fitted monoexponential equation.

was precipitated by addition of 500 µL 10% sulfosalicylic acid to 500 µL plasma, taken at 2, 5, 10, 15, 25, 35, 45, 55 and 65 min after tracer injection and after centrifugation the precipitate ^{11}C-concentration was measured in the well counter. Precipitate radioactivity was corrected for trapped supernatant radioactivity. The supernatant was analyzed for ^{11}C-metabolites by radio-HPLC on a Pnenomenex Sphereclone ODS(2) column (pore size 5 µm) measuring 250 x 4.6 mm (Phenomenex, Torrance, CA, US). The eluent was a mixture of 0.1 M sodium dihydrogen phosphate, 2.6 mM octanesulphonic acid sodium salt and 0.1 mM EDTA, pH adjusted to 3.3 with acetic acid. The flow rate was 2 mL/min, and radioactivity was continuously measured using a NaI scintillation

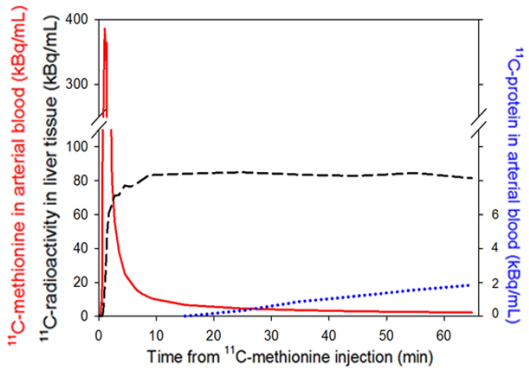

Figure 3. Time courses of ^{11}C-radioactivity concentration in liver tissue (black, dashed), ^{11}C-methionine concentration in arterial plasma (red, solid), and ^{11}C-protein concentration in arterial plasma (blue, dotted). Pig no. 9.

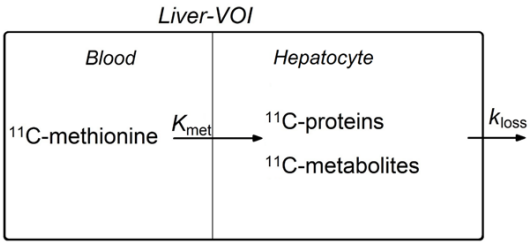

Figure 4. Compartment model of hepatic ^{11}C-methionine metabolism. K_{met}, the hepatic systemic metabolic clearance of ^{11}C-methionine (mL plasma/mL liver tissue/min), k_{loss}, the secretion rate constant of ^{11}C-protein + ^{11}C-metabolites from hepatocytes to blood (min^{-1}).

detector (Gabi, Raytest, Germany) in series to the UV detector. All plasma ^{11}C-concentration measurements were corrected for radioactive decay back to start of the scan.

Plasma ^{11}C-radioactivity concentrations were corrected for average time course of the fractions of ^{11}C-proteins and ^{11}C-metabolites using a fitted monoexponential function (**Figure 2**). **Figure 3** shows an example of the time courses of ^{11}C-radioactivity concentration in liver tissue VOI and plasma concentration of un-metabolized ^{11}C-methionine.

Plasma concentration of unlabeled methionine was determined after precipitation of plasma proteins (see above) by analytical HPLC applying a Phenomenex Sperisorb NH2 column, 250 x 4.6 mm. acetonitrile:water 70:30 as eluent, flow rate 1 mL/min and 230 nm detection wavelength. The concentration of methionine

Table 1. Hepatic metabolism of ^{11}C-methionine in pigs

	K_{met} (mL plasma/mL liver tissue/min)		k_{loss} (min^{-1})		F_{flux} (μmol methionine/mL liver tissue/min)		R_{prot} (kBq/mL plasma/min)	
Procedure	Lap	Pneu	Lap	Pneu	Lap	Pneu	Lap	Pneu
Pig 1 and 2	0.231	0.216	0.0037	0.0056	4.34	1.43	0.085	0.049
Pig 3 and 4	0.122	0.411	0.0028	0.0119	1.80	7.72	0.037	0.083
Pig 5 and 6	0.290	0.175	0.0070	0.0068	5.53	2.98	0.061	0.008
Pig 7 and 8	0.338	0.124	0.0090	0.0043	n.d.	2.02	0.055	0.038
Pig 9 and 10	0.086	0.179	0.0033	0.0011	1.81	4.41	0.021	0.045
Pig 11	0.163		0.0038		3.89		0.051	
Mean	0.205	0.222	0.0049	0.0059	3.47	3.71	0.052	0.045
P	0.81		0.62		0.86		0.64	
Average mean (n = 11)	0.212		0.0054		3.59		0.048	

Lap, laparotomy; Pneu, pneumoperitoneum; K_{met}, hepatic metabolic clearance of ^{11}C-methionine; k_{loss}, rate constant of secretion of ^{11}C-protein and ^{11}C-metabolites from liver tissue to blood; F_{flux}, flux of methionine from plasma to liver tissue; R_{prot}, rate of appearance of ^{11}C-protein in plasma; n.d., not determined.

was calculated by comparison of the area under the methionine peak to the area obtained from a reference solution of methionine with known concentration.

Data analysis

Data was analyzed by an extended Patlak analysis [21] that accounts for irreversible metabolism of ^{11}C-methionine (hepatic systemic metabolic clearance, K_{met}) and loss of metabolized ^{11}C-tracer to blood (rate constant, k_{loss}). The time course of arterial concentration of ^{11}C-methionine was used as input function and the time course of ^{11}C-concentration in liver tissue 15-70 min after tracer injection as output function. For this analysis, a single arterial input can replace dual input from the portal vein and hepatic artery [22]. The compartmental model of the hepatic ^{11}C-methionine metabolism (**Figure 4**), was used to calculate; *i*) the hepatic systemic metabolic clearance of ^{11}C-methionine, K_{met} (mL plasma/mL liver tissue/min) as a measure of intracellular conversion of ^{11}C-methionine to ^{11}C-protein and ^{11}C-metabolites, and *ii*) the secretion rate constant, k_{loss} (min^{-1}), interpreted as the fraction ^{11}C-concentration in the Liver-VOI secreted to blood as ^{11}C-proteins + ^{11}C-metabolites per minute. The appearance rate of ^{11}C-proteins in plasma, R_{prot} (kBq/mL plasma/min), was determined as the slope of the linear regression of the time course of plasma ^{11}C-protein concentration (**Figure 3**). The flux of unlabeled methionine from plasma into liver tissue, F_{flux} (μmol methionine/mL liver tissue/min) was calculated as K_{met} multiplied by plasma methionine concentration (μmol methionine/mL plasma).

Statistics

Differences among the groups of animals were analyzed using unpaired t-test. Pearson product moment correlation coefficient was used to evaluate the correlation between the estimated parameters. A *P*-value of ≤ 0.05 was considered to indicate statistical significance.

Results

The time courses of the radioactivity concentrations in arterial plasma and liver tissue are illustrated in **Figure 3**. The plasma concentration of ^{11}C-methionine showed an initial peak and then decreased to a low level and kept decreasing throughout the experimental period. The ^{11}C-concentration in liver tissue increased rapidly to near steady-state and, after a delay of approx. 17 min, ^{11}C-protein plasma concentration increased linearly.

The extended Patlak model fitted the measurements well with no systematic or significant deviations. There were no statistical significant differences of any of the metabolic parameters between the laparotomy and pneumoperitoneum groups (**Table 1**; every $P > 0.6$). The concentration of unlabeled methionine was also not statistically significantly different between the two groups (average mean; 18.1 μmol methionine/mL plasma, range; 6.6-24.5 μmol methionine/mL plasma, $P = 0.41$). We therefore com-

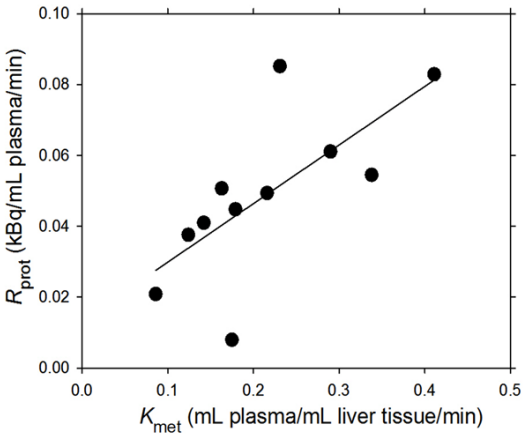

Figure 5. Scatter plot of relation between R_{prot}, appearance rate of ^{11}C-protein in plasma, and K_{met}, hepatic systemic metabolic clearance of ^{11}C-methionine in 11 pigs. Linear regression line is shown. The correlation coefficient, r, is 0.71 (P = 0.015).

bined data from the two groups in the further analysis.

K_{met} was significantly correlated with R_{prot} (P = 0.015) (**Figure 5**), i.e. the hepatic systemic metabolic clearance of ^{11}C-methionine from plasma (measured by PET), was significantly correlated to the appearance rate of ^{11}C-proteins in plasma (measured from blood sampling).

The intercept on the abscissa of the linear regression of the time course of the plasma concentration of ^{11}C-protein was on average 16.7 minutes (range, 9.6-36.2 minutes), reflecting the time delay from tracer injection to appearance of ^{11}C-proteins in plasma, secreted by the liver.

Discussion

The main result of the present study is that the PET-measured hepatic systemic metabolic clearance of ^{11}C-methionine (K_{met}) correlated significantly to the blood-measured appearance rate of ^{11}C-protein in blood (R_{prot}). This suggests that dynamic PET of the liver with ^{11}C-methionine can be used to measure hepatic protein secretion because the liver is the main contributor of plasma proteins. Clinically, plasma albumin concentration is routinely used as a surrogate measure of hepatic protein synthesis but the albumin concentration can be reduced not only in patients with liver disease but also in patients without liver impairment, for example patients with kidney disease. The use of plasma albumin may thus be replaced by dynamic ^{11}C-methionine PET of the liver in clinical situations where a specific measure of the hepatic protein production and secretion is wanted, for example in patients with ascites or after liver transplantation. This is an interesting aspect because other liver tests measure uptake from blood and metabolism [23, 24] or hepatobiliary excretion [25, 26].

The hepatic catabolic stress response, measured by functional hepatic nitrogen clearance, was found significantly higher in pigs exposed to laparotomy compared to pneumoperitoneum [18] which was not confirmed in the present study. We would expect that the laparotomy pigs would have a higher amino acid turn over and thus a higher K_{met} and F_{flux} compared to the pneumoperitoneum pigs. However, an indispensable amino acid, as methionine, might not follow the pattern of higher hepatic metabolism rate during catabolic stress, because the degradation from protein might be down regulated and the body try to preserve methionine instead of elimination trough e.g. transsulfation [3]. This could explain why no significant difference in K_{met} or F_{flux} was found in the present study between pneumoperitoneum and laparotomy. ^{11}C-methionine might thus not be the best tracer to quantify hepatic stress response.

In humans the most abundant metabolites from methionine are 4-methylthio-2-oxobutyrate (deaminated methionine) and serine [10]. We did not perform separate analyses of the amino acids but the input function was corrected for ^{11}C-metabolites. However, any possible uptake of ^{11}C-metabolites by the hepatocytes would imitate ^{11}C-methionine and influence the time course of ^{11}C-concentration in liver tissue (PET) which would result in overestimation of K_{met}. However, if we calculated K_{met} 5-15 min after injection, where only minor amounts of metabolites were present, mean K_{met} 0.237 mL plasma/mL liver tissue/min was not significantly different to the present mean K_{met} 0.212 mL plasma/mL liver tissue/min (paired t-test, P = 0.10). The error from this possible bias is acceptable. In this context, it should be emphasized that if a simple Patlak model without k_{loss} was applied to the data, the difference become significant (paired t-test, P < 0.001, data not

shown) reflecting the importance of k_{loss} in the analysis.

In order to measure plasma ^{11}C-protein for estimation of R_{prot}, we precipitated plasma with sulfosalicylic acid, but a study in mice showed that 19% of metabolites in plasma, 60 minutes after injection of ^{11}C-methionine was actually lipids and derived from the acid precipitable fraction [7]. However, 36% was protein and 45% was other (non-acid precipitable) metabolites. Furthermore, in a human study, incorporation of [methyl-$^{2}H_3$]-methionine into proteins were almost 4 times faster than transmethylation (e.g. for lipid synthesis) in the post absorptive state [3]. The pigs in our study had fasted for 16 hours before the PET examination, so the main radioactivity in the precipitate is thus most likely from ^{11}C-proteins. The ^{11}C-proteins appeared in plasma on average 16.7 min after tracer injection which was comparable to a study in humans [10] and probably due to synthesis and transport of ^{11}C-proteins in the hepatocytes.

In conclusion, we developed a functional PET method for quantification of hepatic ^{11}C-methionine metabolism in pigs and found a significant correlation to the appearance rate of ^{11}C-proteins in plasma. We found no significant difference in metabolism of ^{11}C-methionine between the laparotomy group and the pneumoperitoneum group. Dynamic ^{11}C-methionine PET of the liver may prove clinically useful as a quantitative liver test of the hepatic protein secretion but this need to be confirmed in a human study.

Declaration of conflicts of interest

None.

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