Hepatic sugar phosphate levels reflect gluconeogenesis in lung cancer: simultaneous turnover measurements and $^{31}$P magnetic resonance spectroscopy in vivo

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ABSTRACT

Stable-isotope tracers were used to assess whether levels of phosphomonoesters (PME) and phosphodiesters (PDE) in the livers of lung cancer patients, as observed by $^{31}$P magnetic resonance (MR) spectroscopy, reflect elevated whole-body glucose turnover and gluconeogenesis from alanine. Patients with advanced non-small-cell lung cancer without liver metastases ($n = 24$; weight loss 0–24%) and healthy control subjects ($n = 13$) were studied after an overnight fast. $^{31}$P MR spectra of the liver in vivo were obtained, and glucose turnover and gluconeogenesis from alanine were determined simultaneously using primed-constant infusions of [6,6-$^{2}$H$_2$]glucose and [3-$^{13}$C]alanine. Liver PME concentrations were 6% higher in lung cancer patients compared with controls (not significant); PME levels in patients with $\geq 5\%$ weight loss were significantly higher than in patients with $< 5\%$ weight loss ($P < 0.01$). PDE levels did not differ between the groups. In lung cancer patients, whole-body glucose production was 19% higher (not significant) and gluconeogenesis from alanine was 42% higher ($P < 0.05$) compared with healthy subjects; turnover rates in lung cancer patients with $\geq 5\%$ weight loss were significantly elevated compared with both patients with $< 5\%$ weight loss and healthy subjects ($P < 0.05$). PME levels were significantly correlated with glucose turnover and gluconeogenesis from alanine in lung cancer patients ($r = 0.48$ and $r = 0.48$ respectively; $P < 0.05$). In conclusion, elevated PME levels in lung cancer patients appear to reflect increased glucose flux and gluconeogenesis from alanine. These results are consistent with the hypothesis that elevated PME levels are due to contributions from gluconeogenic intermediates.

INTRODUCTION

Weight loss is a common phenomenon in patients with cancer, and contributes significantly to the high morbidity and mortality in this disease [1–3]. Since it was suggested that anorexia alone cannot fully account for the occurrence of weight loss in subjects with several tumour types, including lung cancer, attempts have been made to investigate underlying mechanisms in the cancer-bearing host. Profound alterations in host metabolism, including

Key words: alanine, gluconeogenesis, liver, lung cancer, $^{31}$P magnetic resonance spectroscopy, stable isotope tracers, weight loss.

Abbreviations: G6P, glucose 6-phosphate; GPC, glycerophosphocholine; GPE, glycerophosphoethanolamine; MR, magnetic resonance; MRS, MR spectroscopy; PC, phosphocholine; PDE, phosphodiester(s); PE, phosphoethanolamine; PEP, phosphoenolpyruvate; PME, phosphomonoester(s); WL, weight-losing.

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elevated protein breakdown [4], increased glucose turnover [5] and endogenous glucose production [6], have been reported, and were suggested to contribute to the observed weight loss in cancer.

It has been argued that the liver may play an important role in the metabolic alterations that contribute to weight loss in cancer [7,8]. Altered enzyme activities [9–12] as well as decreased phosphorylation and energy status [12,13] were reported in the livers of tumour-bearing rats. Increasing tumour burden was shown to be correlated with decreasing phosphorylation status [14] and increasing gluconeogenic enzyme activity in the liver [15]. In contrast, in humans information on liver metabolism in cancer is extremely scarce. In human hepatomas, the activities of pyruvate carboxylase and glucose-6-phosphatase, both key enzymes of gluconeogenesis, were elevated compared to normal liver tissue [16].

In order to understand alterations in liver metabolism in cancer patients, it is essential to obtain information on liver in vivo. Although non-specific changes in 31P magnetic resonance (MR) spectra have been detected in various liver diseases, including primary and secondary hepatic cancer, the systemic effects of cancer on tumour-free host liver have rarely been investigated in humans in vivo. In a previous 31P-MR spectroscopy (MRS) study, markedly elevated levels of phosphomonoesters (PME) and reduced levels of phosphodiesters (PDE) were observed in the tumour-free (i.e. non-metastatic) livers of weight-losing (WL) cancer patients with various tumour types [17].

Since PME and PDE resonances contain contributions from phospholipid intermediates, membrane precursors, and sugar phosphates such as glucose 6-phosphate (G6P), 3-phosphoglycerate and phosphoenolpyruvate (PEP) [18], it has been difficult to interpret changes in 31P MRS-detected components in terms of metabolic alterations. We recently observed elevated glucose turnover and gluconeogenesis from alanine in WL lung cancer patients [19]. Since gluconeogenesis from alanine occurs predominantly in the liver [20], it was suggested that elevated concentrations of gluconeogenic intermediates caused the increased PME levels in these patients. In the present study, 31P MRS of the liver and turnover measurements were combined in order to relate hepatic and systemic alterations in lung cancer patients. The aim was to investigate whether elevated PME and PDE levels in tumour-free liver, as observed with 31P MRS, are correlated with elevated glucose flux and gluconeogenesis from alanine.

METHODS

Subjects
The study was approved by the Medical Ethical Committee of the Erasmus University Medical Center Rotterdam, Rotterdam, The Netherlands. Patients with non-small-cell lung cancer stage IIIA/B or IV (WHO grading system) attending the outpatient department of the University Hospital Rotterdam were recruited. Patients who were in remission or apparently cured were excluded. Additional exclusion criteria were: liver metastases (as determined by CT/ultrasound); metabolic disease; corticosteroid treatment, elective surgery < 3 months before study; chemo- or radio-therapy < 4 weeks before study; alcohol consumption of more than 100 g/week (= 10 glasses); pregnancy; extreme anorexia; artificial weight reduction by dieting. Healthy subjects without weight loss were included as a control group. All participants gave written informed consent for participation in the study.

Experimental design
The subjects were studied in the morning after an overnight fast. A cannula (0.8 mm × 25 mm) was placed in the left cubital vein for the infusion of stable-isotope tracers. An identical cannula was introduced into the contralateral cubital vein for blood sampling. To study gluconeogenesis, a solution was prepared containing D-[6,6-2H2]glucose (98 atom%) and L-[3-13C]alanine (99 atom%) (Mass Trace, Woburn, MA, U.S.A.) in water, and this was sterilized by autoclaving in glass vials. A priming dose of 0.03 mmol/kg D-[6,6-2H2]glucose was administered, followed by a continuous infusion of 0.01 mmol·kg⁻¹·h⁻¹ D-[6,6-2H2]glucose for 90 min. Simultaneously, a priming dose of 0.08 mmol/kg L-[3-13C]alanine was given, followed by a continuous infusion of 0.04 mmol·kg⁻¹·h⁻¹ L-[3-13C]alanine for 90 min. Both tracer solutions were infused using calibrated syringe pumps (Perfusor® fm; Braun).

Venous blood samples were drawn immediately before the isotope infusions were started, and at 10 min intervals after steady-state conditions had been reached during the tracer infusions. Based on observations in our lab and by others [21], a steady state was obtained between 60 and 90 min of tracer infusion. Isotopic enrichments of 1H- and 13C-labelled glucose, and of 13C-labelled alanine, were determined in plasma.

Biochemistry and turnover measurements
Blood samples were collected in tubes containing lithium heparin (Vacutainer®; Becton Dickinson, Meylan Cedex, France) and immediately stored on ice. After centrifugation (10 min, 1200 g, 4 °C), the plasma was collected and stored at −20 °C until analysed. An aliquot of the infusate was analysed to document actual concentrations of the tracers in each study.

Isotopic enrichments were determined using the following procedures. Plasma was deproteinized by adding 0.3 M BaOH (Sigma Diagnostics, St. Louis, MO, U.S.A.)
and 0.3 M ZnSO₄ (Merck, Darmstadt, Germany). After centrifugation (8 min, 15000 g, 4 °C), the supernatant was applied to an ion-exchange column (mixed bed: AG50W-X8 and AG1-X8, 200–400 mesh, 0.2 g of each; Bio-Rad). Glucose and alanine were eluted from the column using water and 4 M NH₄OH (Merck) respectively, and dried under nitrogen. A glucose derivative (aldonitril penta-acetate) was made as described by Varma et al. [22]. An alanine t-butylidimethylsilyl derivate was prepared as described by Chaves Das Neves et al. [23].

Isotopic enrichments were measured by injecting 1 μl samples with a split ratio of 50:1 on to a fused silica capillary column of 25 m × 0.22 mm, coated with 0.11 μm HT5 (SGE, Ringwood, Victoria, Australia). The relative isotopic enrichments of [6,6-²H]glucose and [¹³C]alanine were determined using a Carlo Erba GC8000 gas chromatograph coupled to a Fisons MD800 mass spectrometer (GC-MS) (Interscience B. V., Breda, The Netherlands) in electron-impact ionization mode. The coefficient of variation for enrichment was 0.2 mol% for both [6,6-²H]glucose and [³¹C]alanine, and no concentration effect was observed at this level of enrichment. Ions were selectively detected at mass unit charge (m/z) 187 for natural glucose and at m/z 189 for the deuterated molecule. The isotopic enrichment of [³¹C]alanine was determined at m/z values of 260 and 261 for [¹³C]alanine and [³¹C]alanine respectively [24].

Total enrichment of [¹³C]glucose was measured separately (aldonitril penta-acetate derivation) using a gas chromatograph combustion isotope ratio mass spectrometer (GC-IRMS) (Optima; Micromass UK, Middlewich, Cheshire, U.K.). The [¹³C]glucose enrichment, as atom% excess, was monitored after combustion to CO₂ at mass 44 for carbon-12 and mass 45 for carbon-13.

The whole-body rate of appearance (Ra) of glucose and gluconeogenesis from alanine were calculated during steady state assuming a one-compartment model, as described by Wolfe [25], and were expressed in units of mmol·kg⁻¹·h⁻¹. It was assumed that the dilution of [¹³C]in intracellular pyruvate pools and at oxaloacetate, caused by exchange with the tricarboxylic acid cycle [25], would be similar in lung cancer patients and healthy subjects.

### ³¹P MRS of the liver

Hepatic ³¹P MR spectra were obtained during steady state of the isotope tracers. Spectroscopy studies were performed with a whole-body MR system equipped with a Helicon magnet operating at 2 T (Vision Magnetom; Siemens AG, Erlangen, Germany). A 16 cm-diam. transmit/receive ³¹H/³¹P surface coil was used for magnetic resonance imaging localization, shimming and ³¹P MRS. Elastic bands were used for positioning the coil lateral to the liver in the mid-axillary plane. Field homogeneity achieved in shimming resulted in water peak line widths that were usually less than 40 Hz (± 0.5 p.p.m.). After obtaining an image of the region of interest, a one-dimensional chemical shift imaging sequence was applied on a transverse slice of 4 cm centred on the surface coil and the liver (1 × 4 phase-encoded matrix; field of view 40 × 40 cm²), yielding volumes of 40 × 10 × 4 cm³ [26]. Five spectra were collected with a 640 μs Hanning-sinc shaped radio frequency pulse, resulting in a flip angle of 135 ° in the centre of the coil and 60 ° (weighted average) in the liver volume with a repetition time of 1 s (40 acquisitions). We demonstrated previously [17] that use of repetition times of 1 s and 20 s gives similar differences for PME levels between WL/weight-stable cancer patients and healthy control subjects. Furthermore, saturation at the repetition time of 1 s and the pulse angle of 60 ° used in our study is maximally 15 % for PME (relative to β-ATP) and 30 % for PDE (relative to β-ATP) [26].

Time domain data were Fourier transformed after Gaussian multiplication (centre, 0 ms; width, 30 ms) and phase corrected. Quantification of spectral peak areas was performed using the Numaris-3 software package (Siemens AG), including polynomial baseline correction followed by frequency domain curve fitting [27]. Metabolite concentrations were calculated from peak areas and expressed relative to total MR-detectable phosphate, as described previously [17]. In each experiment the average of five subsequent ³¹P MR spectra was used for calculations.

### Statistics

Results are presented as means ± S.E.M. Differences between group means were compared using Student’s t-test for independent groups. Reported correlations between variables are Pearson’s correlation coefficients. Differences were considered statistically significant at P values of < 0.05.

### RESULTS

A total of 24 patients with non-small-cell lung cancer, stage IIIA/B or IV (WHO grading system), and 13 healthy control subjects were included in the study. In the lung cancer group the mean age was 66 years (range 38–85 years), which was significantly higher than in the control group (46 years; range 25–69 years) (P < 0.05). Body weight was significantly lower in cancer patients than in the healthy controls (65.7 ± 2.5 and 75.3 ± 2.7 kg respectively; P < 0.05). The cancer patients had lost on average 5 ± 1 kg or 7 % (range 0–24 %) of their pre-illness body weight in the previous 6 months.

Whole-body turnover rates measured in lung cancer
patients and healthy controls are shown in Table 1. Since it was shown previously that turnover rates are correlated with the degree of weight loss [28], data for patients with < 5% (weight-stable) or ≥ 5% (WL) weight loss are presented separately, as well as presenting data for all lung cancer patients together. Gluconeogenesis from alanine was significantly higher in lung cancer patients than in control subjects (P < 0.05). Both the rate of turnover of glucose and gluconeogenesis from alanine were significantly elevated in WL lung cancer patients compared with both weight-stable patients and healthy controls (P < 0.01). Age did not significantly influence these results (results not shown).

Table 1  Rate of appearance (turnover) of glucose and of gluconeogenesis from alanine in healthy control subjects and in lung cancer patients

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Glucose turnover (mmol·kg⁻¹·h⁻¹)</th>
<th>Gluconeogenesis from alanine (mmol·kg⁻¹·h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 13)</td>
<td>0.52 ± 0.05</td>
<td>0.28 ± 0.04</td>
</tr>
<tr>
<td>Lung cancer (n = 24)</td>
<td>0.62 ± 0.04</td>
<td>0.41 ± 0.04*†</td>
</tr>
<tr>
<td>&lt; 5% weight loss (n = 12)</td>
<td>0.53 ± 0.05</td>
<td>0.32 ± 0.05*†</td>
</tr>
<tr>
<td>≥ 5% weight loss (n = 12)</td>
<td>0.71 ± 0.06*†</td>
<td>0.51 ± 0.04**††</td>
</tr>
</tbody>
</table>

Since it was shown previously that turnover rates are correlated with the degree of weight loss [28], data for patients with < 5% (weight-stable) or ≥ 5% (WL) weight loss are presented separately, as well as presenting data for all lung cancer patients together. Gluconeogenesis from alanine was significantly higher in lung cancer patients than in control subjects (P < 0.05). Both the rate of turnover of glucose and gluconeogenesis from alanine were significantly elevated in WL lung cancer patients compared with both weight-stable patients and healthy controls (P < 0.01). Age did not significantly influence these results (results not shown).

Examples of MR spectra from a healthy control subject and from both a weight-stable and a WL lung cancer patient are shown in Figure 1. PME were significantly elevated in WL lung cancer patients when compared with weight-stable patients (P < 0.01) (Table 2). No significant differences in PDE levels were observed between the groups. Although PME/PDE ratios were higher in lung cancer patients than in controls, the difference did not reach statistical significance. Again, age did not significantly influence the results.

In Figure 2, correlations between liver metabolites and turnover measurements in lung cancer patients are shown. PME was significantly correlated with both glucose turnover and gluconeogenesis from alanine in lung cancer patients (r = 0.48 and r = 0.48 respectively; P < 0.05). In healthy subjects no significant correlations were observed (r = −0.19 and r = −0.24 respectively; P > 0.42). PDE was not correlated with either glucose turnover or gluconeogenesis from alanine in lung cancer patients (r = −0.30 and r = −0.39 respectively; P > 0.05) or in control subjects (r = −0.04 and r = −0.22 respectively; P > 0.47). Positive correlations between the PME/PDE ratio and glucose turnover or gluconeogenesis from alanine were observed in lung cancer patients (r = 0.47 and r = 0.55 respectively; P < 0.05), but not in healthy controls (r = −0.18 and r = −0.02 respectively).
Table 2  Hepatic metabolite levels as observed by $^{31}$P MRS in healthy control subjects and in lung cancer patients
Metabolite levels are expressed as a proportion of total MR-detectable phosphate (P_total). Weight loss was defined as percentage weight loss from pre-illness stable weight in the 6 months prior to the study. Results are means ± S.E.M. Statistically significant difference from lung cancer patients with < 5% weight loss: * $P < 0.01$ (Student’s t-test for independent groups).

<table>
<thead>
<tr>
<th>Subjects</th>
<th>PME/P_total</th>
<th>PDE/P_total</th>
<th>PME/PDE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 13)</td>
<td>0.079 ± 0.007</td>
<td>0.298 ± 0.018</td>
<td>0.275 ± 0.029</td>
</tr>
<tr>
<td>Lung cancer (n = 24)</td>
<td>0.084 ± 0.005</td>
<td>0.293 ± 0.015</td>
<td>0.319 ± 0.027</td>
</tr>
<tr>
<td>&gt; 5% weight loss (n = 12)</td>
<td>0.069 ± 0.004</td>
<td>0.279 ± 0.024</td>
<td>0.289 ± 0.037</td>
</tr>
<tr>
<td>≥ 5% weight loss (n = 12)</td>
<td>0.098 ± 0.008*</td>
<td>0.306 ± 0.019</td>
<td>0.349 ± 0.040</td>
</tr>
</tbody>
</table>

Figure 2  Metabolite concentrations plotted against flux measurements for lung cancer patients (n = 24)
Metabolite concentrations were measured by $^{31}$P MRS of the liver, and flux measurements were obtained by primed-constant infusion of stable-isotope tracers. P_total, total MR-detectable phosphate.

DISCUSSION

$^{31}$P MRS is a promising tool for the non-invasive study of disease states [29,30]. However, one limitation to its application in human disease has been the difficulty in interpreting $^{31}$P MR spectra in terms of metabolic alterations. To our knowledge, no previous studies combining $^{31}$P MRS with turnover measurements have been reported, in contrast with studies using $^{13}$C MRS [31,32]. In the present study, quantitative information on...
Scheme 1  Pathway of hepatic gluconeogenesis from alanine

Some of the intermediates contributing to the PME and PDE resonances in $^{31}$P MRS spectra are indicated. Numbers represent key gluconeogenic enzymes: 1, pyruvate carboxylase; 2, PEP carboxykinase; 3, fructose-1,6-bisphosphatase; 4, glucose-6-phosphatase.

hepatic substrate metabolism in lung cancer patients was obtained by simultaneous application of $^{31}$P MRS and turnover measurements. Specifically, the relationship between hepatic concentrations of PME and PDE (as observed by $^{31}$P MRS) and glucose turnover and gluconeogenesis from alanine (using stable-isotope tracers) was assessed.

Several authors have used $^{31}$P MRS for the characterization of various disease states in humans and animal models. Elevated PME levels have been reported in diseased liver, for instance in patients with liver cirrhosis [33–36], chronic alcohol abuse [37] or hepatic malignancies [38]. It was suggested that this was caused by contributions of glycerol 3-phosphate or intermediates in the pathway of phospholipid biosynthesis, i.e. phosphocholine (PC) or phosphoethanolamine (PE). Furthermore, $^{31}$P MRS studies revealed reduced hepatic PDE levels in patients with cirrhosis [34–36] or hepatic malignancies [38], and elevated PDE levels in subjects suffering from alcohol abuse, depending on the type and severity of the disease [37]. Decreased levels of glycerophosphocholine (GPC) and glycerophosphoethanolamine (GPE), both products of phospholipid breakdown, or decreased amounts of endoplasmic reticulum within the hepatocytes were suggested as possible contributors to these changes.

In the present study, $^{31}$P MRS was performed in lung cancer patients with healthy livers, thus comprising a distinct metabolic situation. The absence of liver metastases in all cancer patients was verified by CT/ultrasound; furthermore, all patients had normal liver function tests. Hepatic PME levels were increased in lung cancer patients with weight loss, confirming previous studies in patients with mixed tumour types [17], but no difference in PDE levels was observed between lung cancer patients and healthy controls. Even though the mean age of lung cancer patients was higher than that of healthy subjects, the age ranges largely overlapped. Furthermore, we checked our data for significant correlations between metabolite levels and age. No significant correlations were observed between age and PME ($r = 0.16; P = 0.33$), PDE ($r = −0.01; P = 0.98$) or PME/PDE ($r = 0.21; P = 0.22$). In addition, Bourdel-Marchasson et al. [39] reported that hepatic PME and PDE levels and PME/PDE ratios in young healthy subjects (age 30.5 ± 2.1 years) and elderly healthy subjects (age 80.4 ± 6.3 years) were not significantly different.

Glucose turnover and gluconeogenesis from alanine in the present study were elevated in WL lung cancer patients, as reported previously [28].

The pathway of gluconeogenesis from alanine is shown in Scheme 1. In the cytosol, alanine is converted into pyruvate, which in turn is transported into the mitochondria and converted into oxaloacetate by pyruvate carboxylase. Liu et al. [40], using $^{13}$C MRS, suggested that the activity of pyruvate carboxylase was increased in the livers of tumour-bearing rats. The next step in the pathway is the conversion of oxaloacetate into PEP by PEP carboxykinase, a key regulatory enzyme in the gluconeogenic pathway, which in humans is present in the mitochondria and in the cytosol [41]. In rats bearing sarcomas, the activity of PEP carboxykinase was about twice that in healthy rats [9,15], and increased with increasing tumour burden [42]. When PEP enters the gluconeogenic pathway, it is converted into glucose via a number of intermediates, including 3-phosphoglycerate, fructose 1,6-bisphosphate, fructose 6-phosphate and G6P. Two other key enzymes of the gluconeogenic pathway, fructose-1,6-bisphosphatase and glucose-6-phosphatase, catalyse the reactions from fructose 1,6-bisphosphate to fructose 6-phosphate and from G6P to glucose respectively. Elevated activity of glucose-6-phosphatase was detected in the livers of sarcoma-bearing rats, whereas the activity of fructose-1,6-bisphosphatase was only slightly increased [9].
Since the interpretation of PME or PDE concentrations alone is not straightforward, in the present study a comparison with turnover measurements was made. PME was correlated with glucose turnover and with gluconeogenesis from alanine in lung cancer patients, but not in healthy control subjects. The elevated PME levels observed in WL lung cancer patients could be a result of the accumulation of MR-detectable gluconeogenic intermediates within the liver. It has been shown previously that infusion of a gluconeogenic substrate caused \(^{31}\)P MRS-detectable changes in the PME and PDE resonances due to increased concentrations of 3-phosphoglycerate and PEP respectively within the livers of healthy humans and rats [13,43,44]. So far, MRS data on tumour-free livers in humans or animal models are extremely limited. \(^{31}\)P MRS metabolite profiles of biopsy samples of histologically normal liver tissue from patients bearing hepatomas showed elevated contributions of PE and PC; however, changes in sugar phosphate concentrations could not be quantified in that study due to the significant period of ischaemia which arises during the collection of human biopsies [45]. In tumour-free livers from rats bearing the Dunning prostate tumour, decreased levels of G6P and fructose 6-phosphate were reported compared with control rats [13]. It was suggested that the decreased levels of G6P could have been caused by elevated glucose-6-phosphatase activity.

In contrast with PME levels, PDE levels in lung cancer patients in the present study were inversely correlated with gluconeogenesis from alanine, possibly indicating increased conversion of PEP into 2-phosphoglycerate and beyond. PME/PDE ratios were significantly correlated with whole-body glucose turnover and with gluconeogenesis from alanine in lung cancer. We cannot exclude contributions of PC, PE, GPC, GPE or endoplasmic reticulum to the PME and PDE resonances. However, bearing in mind that the livers of the lung cancer patients examined in the present study were apparently healthy (verified by CT/ultrasound and liver function tests), elevated phospholipid turnover would not be likely. Moreover, the elevated PME levels were specific for WL lung cancer patients, being absent in weight-stable patients. There is no hypothesis present in the literature linking PC, PE, GPC or GPE to weight loss in cancer. In contrast, there is a clear hypothesis linking weight loss and elevated gluconeogenesis in cancer. Therefore it seems unlikely that elevated PME and PDE signals were caused by PC, PE, GPC or GPE. The fact that in healthy control subjects hepatic PME levels are not significantly correlated with glucose flux or gluconeogenesis would suggest that metabolites other than gluconeogenic intermediates, such as PC and PE, are the predominant contributors to the PME resonance in these subjects.

In summary, this combined turnover and \(^{31}\)P MRS study in humans \textit{in vivo} suggests that elevated hepatic PME levels in lung cancer patients reflect increased glucose flux and gluconeogenesis from alanine in these patients. Our data are consistent with the hypothesis that elevated PME is due to contributions from gluconeogenic intermediates, possibly 3-phosphoglycerate. In healthy controls no such correlation was observed. Further studies will need to address whether \(^{31}\)P MRS has predictive value for the subsequent development of weight loss in cancer patients.

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