

Altered Hepatic Gluconeogenesis during L-Alanine Infusion in Weight-losing Lung Cancer Patients as Observed by Phosphorus Magnetic Resonance Spectroscopy and Turnover Measurements¹

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ABSTRACT

Profound alterations in host metabolism in lung cancer patients with weight loss have been reported, including elevated phosphomonoesters (PMEs) as detected by ³¹P magnetic resonance spectroscopy (MRS). In healthy subjects, infusion of L-alanine induced significant increases in hepatic PMEs and phosphodiester (PDEs) due to rising concentrations of 3-phosphoglycerate and phosphoenolpyruvate, respectively. The aim of the present study was to monitor these changes in the tumor-free liver of lung cancer patients during L-alanine infusion by means of simultaneous ³¹P MRS and turnover measurements. Twenty-one lung cancer patients without liver metastases with (CaWL) or without weight loss (CaWS), and 12 healthy control subjects were studied during an i.v. L-alanine challenge of 1.4–2.8 mmol/kg followed by 2.8 mmol/kg/h for 90 min. Plasma L-alanine concentrations increased during alanine infusion, from 0.35–0.37 mM at baseline to 5.37 ± 0.14 mM in the CaWL patients, 6.67 ± 0.51 mM in the CaWS patients, and 8.47 ± 0.88 mM in the controls (difference from baseline and between groups during alanine infusion, all *P* < 0.001). Glucose turnover and liver PME levels at baseline were significantly elevated in the CaWL patients. Alanine infusion increased whole-body glucose turnover by 8 ± 3% in the CaWS patients (*P* = 0.03), whereas no significant change occurred in the CaWL and controls. PME levels increased by 50 ± 16% in controls (area under the curve, *P* < 0.01) and by 87 ± 31% in the CaWS patients (*P* < 0.05) after 45–90 min. In contrast, no significant changes in PME levels were observed in the CaWL patients. Plasma insulin concentrations increased during L-alanine infusion in all groups to levels that were lower in the CaWL patients than in the CaWS patients and controls (*P* < 0.05). In lung cancer patients, but not in controls, changes in PME and PDE levels during alanine infusion were inversely correlated with their respective baseline levels (*r* = −0.82 and −0.86, respectively; *P* < 0.001). In addition, changes in PMEs during alanine infusion in lung cancer patients were inversely correlated with the degree of weight loss (*r* = −0.54; *P* < 0.05). This study demonstrates the presence of major alterations in the pathway of hepatic gluconeogenesis in weight-losing lung cancer patients, as shown by elevated glucose flux before and during L-alanine infusion, and by the increased PME and PDE levels, which reflect accumulation of gluconeogenic intermediates in these patients. Weight-stable lung cancer patients show accelerated increases in PME and PDE levels during L-alanine infusion, suggesting enhanced induction of the gluconeogenic pathway. Our results suggest altered gluconeogenic enzyme activities and elevated alanine uptake within the livers of weight-losing/weight-stable lung cancer patients.

INTRODUCTION

Weight loss in lung cancer is associated with both impaired therapy outcome (1) and reduced survival (1–4). Characteristic features of weight loss in lung cancer are breakdown of both fat mass and skeletal muscle, whereas visceral organs typically are spared or even enlarged

(5, 6). Although profound alterations in host substrate metabolism in cancer patients have been reported, mechanisms responsible for the observed weight loss are as yet poorly understood. Isotope tracer studies showed elevated protein breakdown and glucose turnover in lung cancer patients (7–9). Increased gluconeogenesis from alanine was observed in tumor-influenced hepatocytes (10), in tumor-bearing animals *in vivo* (11), and in cancer patients with various tumor types (12). We recently reported increased whole-body gluconeogenesis from alanine in lung cancer patients with weight loss (13). A significant correlation between gluconeogenesis from alanine and the degree of weight loss was also observed.

Because the liver is the main site for gluconeogenesis from alanine (14), the observed increase in gluconeogenesis from alanine as observed in weight-losing lung cancer patients is likely to be partly related with altered liver metabolism (15, 16). In animal models, altered hepatic enzyme activities (17, 18) and decreased liver phosphorylation status (19) and energy balance (18) were correlated with tumor burden (20). Furthermore, elevated concentrations of gluconeogenic intermediates such as glucose-6-phosphate were observed within the livers of these animals (18). Another experimental study revealed altered hepatic metabolism in response to fructose infusion, even in rats with minimal tumor burden (21). These alterations preceded the onset of cachexia, and it was suggested that they were related to elevated hepatic gluconeogenesis in these animals.

Because of a lack of noninvasive techniques, data on altered liver metabolism in humans with lung cancer are limited. In recent studies using ³¹P MRS,³ elevated concentrations of PMEs were observed in the livers of weight-losing cancer patients with various tumor types (22) and lung cancer (23). In contrast, liver PME levels in weight-stable cancer patients were not significantly different from those in healthy subjects. Furthermore, hepatic PME levels were significantly correlated with the rate of gluconeogenesis from alanine in lung cancer patients, but not in healthy subjects (23). MRS studies have also been used to obtain dynamic information on liver metabolism by monitoring changes in hepatic metabolite concentrations during infusion of a gluconeogenic substrate. Changes in PME and ATP levels were reported in studies using ³¹P MRS with L-alanine infusion *in vivo* in healthy rats (24) as well as in rats after ischemia (25) or surgery (26). In healthy humans, ³¹P MRS with either a bolus (27) or continuous (28) infusion of L-alanine has been shown to provide information on changes in concentrations of gluconeogenic intermediates within the liver. However, information on liver gluconeogenic intermediates during a metabolic challenge in lung cancer patients is lacking.

The aims of the present study were to compare glucose metabolism in the tumor-free livers of weight-losing and weight-stable lung cancer patients and healthy subjects by means of ³¹P MRS, with infusion of L-alanine as a gluconeogenic substrate. Data were compared with

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³ The abbreviations used are: MRS, magnetic resonance spectroscopy; PME, phosphomonoester; AUC, area under the curve; CaWL, lung cancer with weight loss; CaWS, lung cancer with weight stable; PDE, phosphodiester; 3PG, 3-phosphoglycerate.

flux measurements, using stable isotope tracers before/during alanine infusion.

MATERIALS AND METHODS

Subjects. The study was approved by the Medical Ethics 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, The Netherlands, were recruited. Patients who were in remission or apparently cured were excluded. Additional exclusion criteria included liver metastases (as checked for by computed tomography/ultrasound), metabolic disease, corticosteroid treatment, elective surgery <3 months prior to the study, chemo- or radiotherapy <4 weeks prior to the study, alcohol consumption of >100 g/week (10 glasses), pregnancy, and extreme anorexia or artificial weight reduction by dieting. Healthy subjects without weight loss were included as a control group. All participants signed informed consent.

Experimental Design. All subjects kept a dietary record during 7 days and refrained from alcoholic drinks for 3 days prior to the MRS measurements. Data on pre-illness stable weight, current weight, and weight loss over the previous 6 months were taken from hospital records supplemented with oral information from patients. The subjects were studied between 7:30 a.m. and 1:00 p.m. after an overnight fast (12–14 h). Body weight was measured to the nearest 0.1 kg on an electrical weighing scale (Seca 707; Hamburg, Germany), height was measured to the nearest 0.1 cm, and the thicknesses of four skinfolds (triceps, biceps, subscapular, and supra iliac) were measured to the nearest 0.2 mm, using a standard skinfold caliper (Holtain Ltd., London, United Kingdom). A cannula (0.8 × 25 mm) was placed in the left cubital vein for the infusion of the stable isotope tracer and unlabeled L-alanine. In the contralateral cubital vein, an identical cannula was positioned for blood sampling. To determine whole-body glucose turnover, a solution was prepared containing [6,6-²H₂]-D-glucose, 98 atom% (Mass Trace, Woburn, Massachusetts) in water, sterilized by autoclaving in glass vials. A solution of (unlabeled) L-alanine in water (100 g/l; Bufa B.V., Uitgeest, The Netherlands) was prepared, sterilized by autoclaving in glass bottles, and warmed to ~30°C.

The study consisted of two phases. During the first phase (baseline), a priming dose of 0.03 mmol/kg of [6,6-²H₂]-glucose was administered, followed by a continuous infusion of 0.01 mmol/kg/h for 90 min. During the second phase, a priming dose of 1.4–2.8 mmol/kg unlabeled L-alanine was administered in 5–8 min, followed by a continuous infusion of 2.8 mmol/kg/h L-alanine for 90 min to reobtain a steady state; simultaneously, the isotope tracer infusion was continued. Venous blood samples were collected as follows: phase 1, one sample immediately before the isotope tracer infusion was started (for determination of background enrichment of ²H-glucose) and at 10-min intervals from 30 to 90 min during isotope tracer infusion, after steady

state was reached; phase 2, at 15-min intervals during continuous L-alanine infusion. Phosphorus MR spectra of the liver were obtained at baseline and at 3-min intervals during L-alanine infusion.

³¹P MRS of the Liver. 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 diameter transmit/receive ¹H/³¹P surface coil was used for MR 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 <40 Hz (0.5 ppm). After an image of the region of interest was obtained, a one-dimensional chemical shift imaging sequence was applied on a transverse slice of 4 cm centered 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 (29). Spectra were collected with a 640-μs Hanning-sinc-shaped radio frequency pulse, resulting in a flip angle of 135 degrees in the center of the coil, and 60 degrees (weighted average) in the liver volume with a repetition time of 1 s (40 acquisitions).

Time domain data were Fourier transformed after gaussian multiplication (center, 0 ms; width, 30 ms) and phase corrected. Quantification of spectral peak areas was performed using a Numaris-3 software package (Siemens AG, Erlangen, Germany), including polynomial baseline correction followed by frequency domain curve fitting (30). Metabolite concentrations were calculated from peak areas and expressed relative to total MR-detectable phosphate as described previously (22). Total MR-detectable phosphate did not change during L-alanine infusion (data not shown).

Substrate Concentrations and Glucose Turnover. Blood samples were collected in tubes containing lithium heparin (Vacutainer; Becton Dickinson, Meylan Cedex, France) and stored immediately on ice. After centrifugation (10 min, 1200 × g, 4°C), the plasma was collected and stored at –20°C until analysis. Blood glucose concentrations were measured enzymatically with a glucose-oxidase/peroxidase assay system (Boehringer Mannheim, Mannheim, Germany). Plasma alanine concentrations were determined enzymatically as described by Williamson (31). Isotopic enrichment of deuterium-glucose (mole percent excess) in plasma was determined by gas chromatography-mass spectrometry as described previously (13). Plasma concentrations of insulin and glucagon were determined at two time points during baseline and two time points during L-alanine infusion by radioimmunoassay techniques (Biosource, Fleurus, Belgium, and Euro-Diagnostica, Sweden, respectively).

Statistical Analysis. Results are reported as means ± SE. In each experiment, the mean of five subsequent MR spectra was used as baseline value for calculations. Mid-time points of MRS data acquisition at 15-min intervals during L-alanine infusion (e.g., 7.5, 22.5, and 37.5 min) were used for graphical representation, with values being expressed relative to the baseline value of healthy control subjects (100%). As a measure of overall spectral response,

Table 1 Characteristics^a of study population

	Lung cancer group			P	
	Control (n = 12)	CaWS (n = 12)	CaWL (n = 9)	Cancer vs. controls	CaWL vs. CaWS
Age (years)	37–69 ^b	38–76	53–81	<0.01	0.20
Gender (M/F)	2/10	7/5	7/2		
Disease stage (IIIA/IIIB/IV)		1/5/6	3/2/4		
Previous therapy (n) ^c					
Surgery	0	2	2		
Radiotherapy	0	6	4		
Chemotherapy	0	6	0		
Weight (kg)	77.0 ± 3.0	72.8 ± 3.4	60.9 ± 3.5	0.36	0.02
Weight change (kg)	0	1 ± 1	–9.0 ± 1.4	0.56	<0.001
Weight change (%)	0	1 ± 1	–12 ± 2	0.59	<0.001
% Ideal body weight	124 ± 5	117 ± 5	96 ± 5	0.33	<0.01
Body mass index (kg/m ²)	25.8 ± 1.0	25.1 ± 1.0	20.9 ± 1.1	0.62	0.01
Sum of skinfolds (mm) ^d	71 ± 6	63 ± 6	30 ± 4	0.29	<0.001
Albumin (g/l)	46 ± 1 ^e	43 ± 1 ^e	38 ± 2 ^e	0.34	<0.01
Prealbumin (g/l)	0.30 ± 0.01	0.29 ± 0.01 ^e	0.17 ± 0.02 ^e	0.47	<0.001

^a Mean ± SE.

^b Range.

^c Antitumor treatment: surgery >3 months, or radio- and chemotherapy ≥4 weeks prior to the MRS study (number of patients).

^d Sum of four skinfolds: biceps, triceps, supra iliac, subscapular (mm).

^e One value missing.

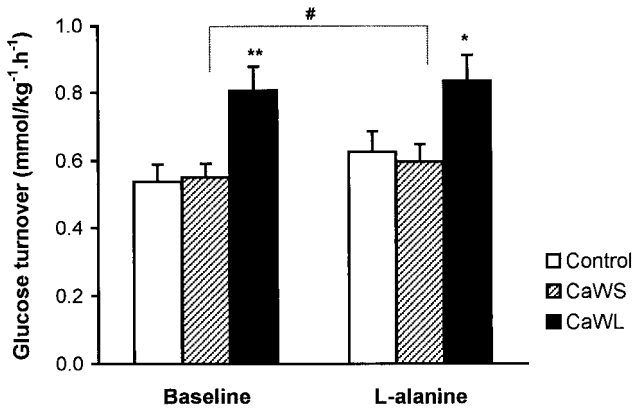


Fig. 1. Whole-body rate of appearance (turnover) of glucose before and during primed-continuous L-alanine infusion in healthy control subjects (Control; $n = 10$) and weight-stable (CaWS; $n = 12$) and weight-losing (CaWL; $n = 6$) lung cancer patients. Turnover rates were assessed using a primed-constant infusion of $[6,6\text{-}^2\text{H}_2]$ -glucose. Results are presented as means (columns); bars, SE. Results for CaWL patients were significantly different from CaWS patients and controls: *, $P < 0.05$; **, $P < 0.01$ (ANOVA, adjusted for age and gender). #, significantly different from baseline: $P < 0.05$ (paired t test).

integrals of time-response curves (AUC) of peak areas over the 0–45, 45–90, and 0–90 min intervals during L-alanine infusion were calculated and expressed relative to the baseline values. Between-group differences in baseline values and response to alanine infusion were analyzed using ANOVA. Changes from baseline values were analyzed using Student's paired t test. Differences between groups were analyzed by multiple regression analysis, using age, gender, and priming dose of L-alanine as covariates. Pearson's correlation coefficients were calculated between baseline metabolite concentrations (expressed relative to total MR-detectable phosphate) and absolute metabolite change (AUC) per minute during L-alanine infusion. Statistical significance was set at $P < 0.05$.

RESULTS

Study Population. Twenty-one patients with non-small cell lung cancer were included in the study: 9 weight-losing ($\geq 5\%$ weight loss; CaWL patients) and 12 weight-stable patients ($< 5\%$ weight loss; CaWS patients). Twelve healthy subjects were included as controls. Characteristics of the study population are listed in Table 1. The mean age of the lung cancer patients was higher than in controls, although age ranges largely overlapped. The disease stage was similar in the CaWL and CaWS patients. The previous antitumor treatment was also comparable in both groups, except for chemotherapy, which had been given as a previous treatment in six CaWS patients but in none of the CaWL patients. Note that none of the patients received any antitumor therapy at the time of the study. The CaWL patients had lost 9.0 ± 1.4 kg (mean \pm SE) or 12% (range, 6–22%) of their pre-illness stable body weight within the 6 months preceding the study. Body weight, body mass index, and sum of skinfolds were significantly lower in the CaWL patients compared with the CaWS patients and controls ($P < 0.05$). Albumin and prealbumin levels were also significantly decreased in CaWL patients. Liver function tests were normal in all subjects. All patients had a history of smoking, compared with 42% of the healthy subjects. Thirty-eight percent of the CaWL patients, 33% of the CaWS patients, and 33% of healthy control subjects were actual smokers at the time of study. No differences in energy intake were detected between any of the groups. Because of the differences in age and gender between the groups, all data were checked for potential confounding by age or gender. Although in no case significant was confounding by age nor gender observed, all presented statistical analyses are adjusted for age and gender.

Plasma Substrate Concentrations and Flux Measurements. Fasting blood glucose levels were similar in lung cancer patients (CaWL patients, 5.8 ± 0.3 mM; CaWS patients, 5.3 ± 0.2 mM) and healthy subjects (5.7 ± 0.2 mM) and did not change during L-alanine infusion (CaWL patients, 5.7 ± 0.4 mM; CaWS patients, 5.0 ± 0.1 mM; controls, 5.5 ± 0.2 mM). Baseline plasma alanine concentrations were similar between lung cancer patients and healthy controls ($0.35\text{--}0.37$ mM). L-Alanine infusion caused a sharp and highly significant rise in plasma alanine concentrations to a mean of 5.37 ± 0.14 mM in the CaWL patients, 6.67 ± 0.51 mM in the CaWS patients, and 8.47 ± 0.88 mM in the controls (CaWL versus CaWS and CaWS versus controls, $P < 0.001$). These postalanine plasma concentrations were significantly different between all groups ($P < 0.001$). Turnover rates of glucose at baseline and during L-alanine infusion are presented in Fig. 1. Whole-body glucose turnover at baseline was 35% higher in the CaWL patients compared with both the CaWS patients and controls ($P < 0.01$). Although during alanine infusion values of glucose turnover appeared to increase in all groups, this was only statistically significant in the CaWS group (0.05 ± 0.02 mmol/kg/h; $P < 0.05$). Glucose turnover during alanine infusion was still 36% higher in CaWL patients than in CaWS patients and controls ($P < 0.05$).

Hepatic Concentrations of Gluconeogenic Intermediates. Baseline PME were significantly elevated in CaWL patients ($10.5 \pm 1.0\%$) compared with CaWS patients and controls ($6.7 \pm 0.5\%$ and $7.9 \pm 0.7\%$, respectively; $P < 0.01$, corrected for

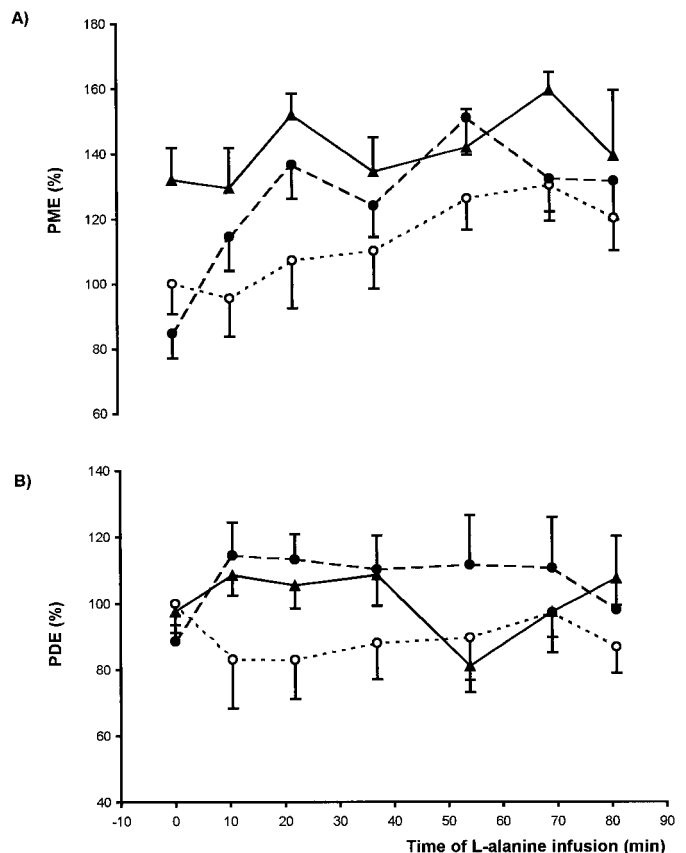


Fig. 2. PME (A) and PDE (B) concentrations in the livers of healthy control subjects (\circ ; $n = 9$) as well as weight-stable (\bullet ; $n = 10$) and weight-losing (\blacktriangle ; $n = 7$) lung cancer patients during a primed-constant infusion of L-alanine (initial priming, 1.4–2.8 mmol/kg; infusion, 2.8 mmol/kg/h). Curves represent means; bars, SE. Baseline values are means of five spectra acquired in each subject before L-alanine infusion. Values are expressed as percentage of mean baseline value of healthy subjects (100%). Times during L-alanine infusion are mid-time points of ^{31}P MRS data collection referenced to the start of the L-alanine infusion ($0 =$ baseline).

Table 2 Hepatic phosphorus metabolite levels^a after primed-constant infusion of L-alanine in healthy control subjects and lung cancer patients

	Time of alanine infusion	Control (n = 9)	Lung cancer		<i>P</i> ^b	
			CaWS (n = 10)	CaWL (n = 7)	Cancer vs. controls	CaWL vs. CaWS
PME	0–45 min	21 ± 9	58 ± 14 ^c	7 ± 8	0.31	0.02
	45–90 min	50 ± 16 ^c	87 ± 31 ^d	7 ± 9	0.55	0.22
	0–90 min	33 ± 11 ^d	69 ± 19 ^c	11 ± 10	0.35	0.28
PDE	0–45 min	-5 ± 7	32 ± 20	23 ± 15	0.56	0.23
	45–90 min	-3 ± 10	40 ± 33	9 ± 12	0.49	0.24
	0–90 min	-8 ± 5	30 ± 22	12 ± 11	0.46	0.32

^a Area under the curve during L-alanine infusion expressed as change from baseline (% , mean ± SE).

^b For between-group differences in response to alanine infusion adjusted for age, gender, and L-alanine prime dose.

^{c,d} Difference from baseline values (Student's paired *t* test): ^c *P* < 0.01; ^d *P* < 0.05.

age), as reported previously. No differences in PDEs (CaWL group, 30.6 ± 1.9%; CaWS group, 27.8 ± 2.2%; controls, 31.4 ± 2.0%) were observed between the groups.

In Fig. 2, changes in hepatic metabolite concentrations during L-alanine infusion are shown. PMEs increased gradually in the controls and reached statistical significance at 60 min (*P* < 0.01). In CaWS patients, PMEs showed a sharp and highly significant rise during the first 30 min and were still significantly elevated at 75 min of L-alanine infusion (*P* < 0.05). The slope of the PME concentration from 0 to 30 min was significantly steeper in CaWS patients than in controls (2.48 ± 0.50%/min versus 0.77 ± 0.45%/min; *P* = 0.01). In contrast, PME concentrations in CaWL patients did not change significantly during alanine infusion. PDEs initially decreased in controls but increased in both CaWS and CaWL patients, with significantly different slopes for the PDE curves between 0 and 30 min for the CaWL and CaWS patients versus healthy subjects (0.62 ± 0.33%/min and 1.20 ± 0.77%/min versus -0.57 ± 0.33%/min, respectively; *P* = 0.02). PDE levels as such were not significantly different from baseline at any time point in any of the three groups.

Overall Changes during L-Alanine Infusion: AUC. Overall changes in metabolite concentrations during L-alanine infusion relative to baseline are presented in Table 2. During the first 45 min of alanine infusion, the increase in PME concentrations was significantly less in CaWL patients than in CaWS patients and controls (*P* = 0.02). At 45–90 min of alanine infusion, this difference in response between the CaWL and CaWS patients and controls remained, although it was no longer statistically significant.

Hormone Levels. Plasma insulin and glucagon levels are presented in Fig. 3. Baseline insulin concentrations were lower in CaWL patients than in the CaWS patients and controls (*P* < 0.05). Insulin levels showed a strong increase at 45 min of L-alanine infusion (*P* < 0.01) and were still significantly elevated from baseline at 90 min in all groups (*P* < 0.01). In CaWL patients, insulin levels remained significantly lower than in both the CaWS and control groups during the 90 min of alanine infusion (*P* < 0.01). Baseline glucagon levels were similar in all groups. L-Alanine infusion caused a substantial rise in plasma glucagon at 45–90 min in all groups (*P* < 0.01). No significant differences in glucagon concentrations during alanine infusion were observed between any of the groups.

Correlations. Spectral changes in PMEs and PDEs during alanine infusion were strongly dependent on their respective baseline concentrations in lung cancer patients (*r* = -0.82 and -0.86, respectively; *P* < 0.001) but not in controls (*r* = -0.07 and -0.30, respectively; Fig. 4). Furthermore, patients with a higher degree of weight loss showed smaller increases in PME levels during alanine infusion (*r* = -0.54; *P* < 0.05).

DISCUSSION

In the present study, hepatic gluconeogenesis from alanine in lung cancer patients was monitored by means of ³¹P MRS during an i.v. L-alanine challenge, and information on gluconeogenic intermediates was obtained noninvasively. Simultaneously, glucose turnover before and during L-alanine infusion was measured using stable isotope tracers.

Glucose flux was significantly elevated in CaWL patients at baseline compared with CaWS patients and control subjects, confirming other studies (7, 13). Changes in glucose turnover during L-alanine infusion were minimal, as could be expected in view of the autoregulatory mechanisms that control hepatic glucose output (32–34). Liver PME levels increased during alanine infusion in both CaWS patients and controls, confirming studies performed in healthy animals (24)

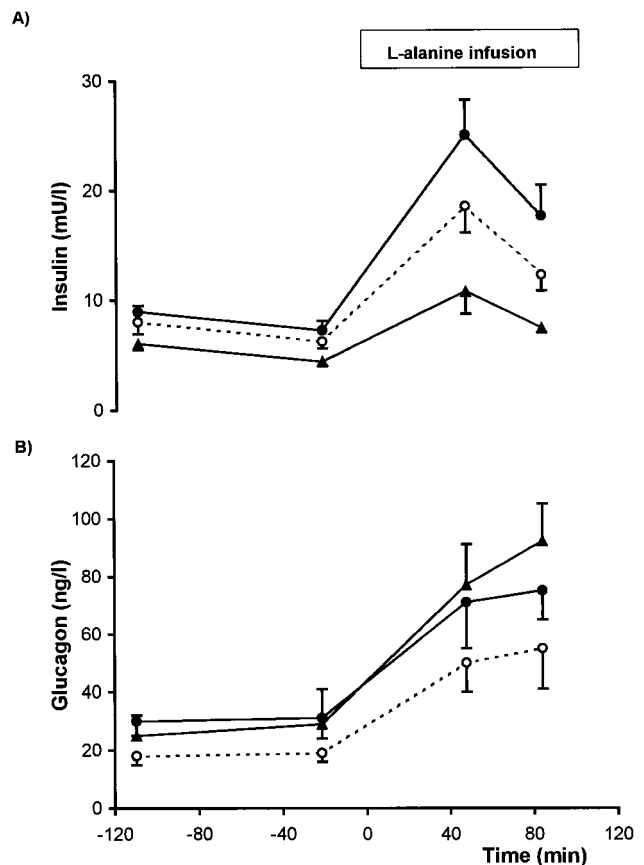


Fig. 3. Plasma insulin (A) and glucagon (B) concentrations in healthy control subjects (○; n = 10), weight-stable (●; n = 10) and weight-losing (▲; n = 6) lung cancer patients before and during a primed-constant infusion of L-alanine (initial priming, 1.4–2.8 mmol/kg; infusion, 2.8 mmol/kg/h). Curves represent means; bars, SE.

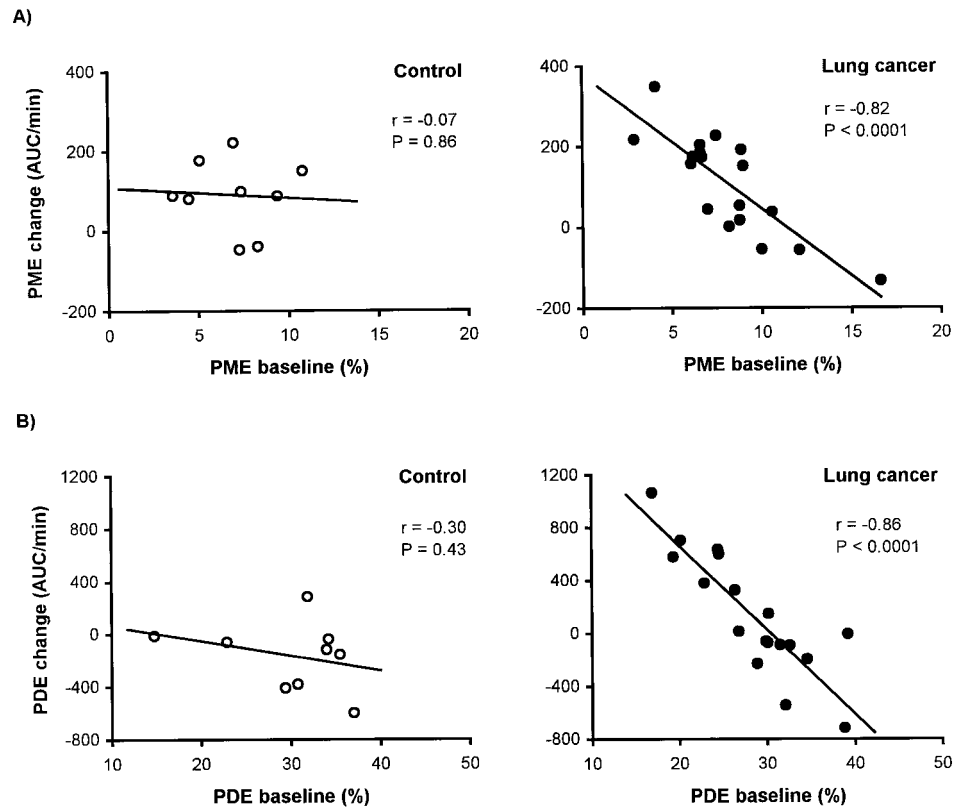


Fig. 4. Changes in PME (A) and PDE (B) concentrations in the livers of healthy control subjects ($n = 9$) and lung cancer patients ($n = 17$) during a primed-constant infusion of L-alanine. Changes plotted against respective baseline values, expressed relative to total MR-detectable phosphate (%). r , Pearson's correlation coefficient.

and humans (27, 28), in which this rise in PMEs was attributed to increased concentrations of 3PG (24, 27). Our finding in the present study that in CaWS patients, PMEs increased significantly faster and reached levels twice as high as in healthy subjects may reflect a more rapid rise in concentrations of 3PG in the livers of these patients. In contrast, in CaWL patients, PME levels were already elevated at baseline and did not increase any further during alanine infusion. Moreover, a strong negative correlation between baseline PME levels and the increase in PMEs during alanine infusion was observed in lung cancer patients, but not in healthy controls, suggesting that the 3PG levels in CaWL patients were maximal at baseline and could not be increased any further by an i.v. alanine challenge.

Although mean PDE concentrations were similar in lung cancer patients and healthy controls both at baseline and during alanine infusion, a significant difference in slope between patients (increase) and healthy subjects (decrease) was detected in the first 30 min of alanine infusion. As for PMEs, changes in PDEs during alanine infusion were negatively correlated with baseline PDE levels in lung cancer patients but not in healthy subjects. The PDE resonance contains components of phospholipid membranes, such as glycerophosphorylethanolamine and glycerophosphorylcholine, and the gluconeogenic intermediate, phosphoenolpyruvate (35). In liver extracts of healthy rats, post-alanine infusion phosphoenolpyruvate concentrations were significantly elevated (24, 27), suggesting that the increase in PDE in lung cancer patients observed in the present study is most likely due to elevated accumulation of phosphoenolpyruvate.

The mechanisms involved in the increasing levels of PME before and during alanine infusion in lung cancer patients could be enhanced uptake of alanine within the hepatocytes and/or elevated gluconeogenic enzyme activity. In animal studies *in vivo*, alanine concentrations in the livers of tumor-bearing hosts were elevated (36), whereas plasma alanine concentrations were decreased (37), suggesting elevated uptake of alanine by the liver. Some authors reported reduced

plasma alanine concentrations in weight-losing lung cancer patients (38, 39). In the present study, we did not detect any differences in baseline plasma alanine levels between the two groups of lung cancer patients and healthy subjects. This indicates that the elevated alanine flux reported previously in weight-losing lung cancer patients (13) is counterbalanced by increased alanine uptake in the liver, resulting in similar plasma levels in CaWL and CaWS patients, and controls. It is noteworthy that although plasma alanine concentrations increased significantly in all groups during infusion of L-alanine, they did not increase to the same extent in all groups, but in the order CaWL < CaWS < controls ($P < 0.001$). Because all statistical analyses were adjusted for alanine priming dose, these differences were not explained by the alanine priming dose. This would imply that alanine uptake by the liver during alanine infusion is increased in lung cancer patients, especially in weight-losing patients.

Elevated activities of gluconeogenic key enzymes in the livers of tumor-bearing hosts have been reported by several authors, which could explain the elevated PME levels in CaWL patients at baseline, as well as the faster and larger increase in PMEs observed in CaWS patients during alanine infusion. Increased PDE and PME levels during alanine infusion, most likely reflecting elevated phosphoenolpyruvate and 3PG concentrations, could be explained by enhanced activities of pyruvate carboxylase (converting pyruvate into oxaloacetate) and/or phosphoenolpyruvate carboxykinase (converting oxaloacetate into phosphoenolpyruvate). The observed increase in glucose production could also be the result of enhanced glucose-6-phosphatase activity. Indeed, animal studies showed elevated activities of pyruvate carboxylase in the livers of rats bearing mammary adenocarcinomas (40), and phosphoenolpyruvate carboxykinase (41) and glucose-6-phosphatase (42) in the livers of sarcoma-bearing rats. Factors that may be involved in the enhanced activities of gluconeogenic enzymes are decreased insulin or increased glucagon concentrations (37, 43, 44), which would stimulate gluconeogenic key en-

zymes (45). Relatively higher glucagon:insulin ratios were observed in CaWL patients compared with CaWS patients.

In summary, this study demonstrates the presence of major alterations in gluconeogenesis in the tumor-free livers of lung cancer patients both with and without weight loss. Weight-losing lung cancer patients have markedly elevated glucose flux before as well as during L-alanine infusion. This is also confirmed by elevated PME and PDE levels within the liver, which reflect accumulation of gluconeogenic intermediates in these patients both before and during alanine infusion. Neither glucose flux nor the concentrations of gluconeogenic intermediates within the liver showed any change during alanine infusion in weight-losing cancer patients, suggesting that gluconeogenesis is already maximally induced at baseline in these patients. Weight-stable lung cancer patients, having a normal glucose flux, showed an accelerated rise in PME and PDE levels during L-alanine infusion. Our results suggest that both altered gluconeogenic enzyme activities within the liver and elevated alanine uptake are involved in these abnormalities.

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