

# Weight loss and elevated gluconeogenesis from alanine in lung cancer patients<sup>1-4</sup>

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## ABSTRACT

**Background:** The role of gluconeogenesis from protein in the pathogenesis of weight loss in lung cancer is unclear.

**Objective:** Our aim was to study gluconeogenesis from alanine in lung cancer patients and to analyze its relation to the degree of weight loss.

**Design:** In this cross-sectional study, we used primed-constant infusions of [6,6-<sup>2</sup>H<sub>2</sub>]-D-glucose and [3-<sup>13</sup>C]-L-alanine to assess whole-body glucose and alanine turnover and gluconeogenesis from alanine in weight-losing (WL, *n* = 9) and weight-stable (WS, *n* = 10) lung cancer patients and healthy control (*n* = 15) subjects.

**Results:** Energy intake and plasma alanine concentrations did not differ significantly among the subject groups. Mean ( $\pm$ SEM) whole-body glucose production was significantly higher in WL than in WS and control subjects ( $0.74 \pm 0.06$  compared with  $0.55 \pm 0.04$  and  $0.51 \pm 0.04$  mmol·kg<sup>-1</sup>·h<sup>-1</sup>, respectively, *P* < 0.01). Alanine turnover was significantly elevated in WL compared with WS and control subjects ( $0.57 \pm 0.04$  compared with  $0.42 \pm 0.05$  and  $0.40 \pm 0.03$  mmol·kg<sup>-1</sup>·h<sup>-1</sup>, respectively, *P* < 0.01). Gluconeogenesis from alanine was significantly higher in WL than in WS and control subjects ( $0.47 \pm 0.04$  compared with  $0.31 \pm 0.04$  and  $0.29 \pm 0.04$  mmol·kg<sup>-1</sup>·h<sup>-1</sup>, respectively, *P* < 0.01). The degree of weight loss was positively correlated with glucose and alanine turnover and with gluconeogenesis from alanine (*r* = 0.45 for all, *P* < 0.01).

**Conclusions:** Aberrant glucose and alanine metabolism occurred in WL lung cancer patients. These changes were related to the degree of weight loss and not to the presence of lung cancer per se. *Am J Clin Nutr* 2000;71:583-9.

**KEY WORDS** Weight loss, gluconeogenesis, alanine, lung cancer, stable isotope tracer, humans, liver, glucose metabolism, alanine metabolism, cachexia

## INTRODUCTION

Weight loss is frequently observed in patients with cancer. Evidence shows that weight loss in such patients is associated with poor treatment outcome and reduced survival (1-3). Weight loss may occur in the early stages of cancer, even before there are other signs that a tumor is present. Although animal studies suggest that the development of weight loss in cancer is closely related to both the presence and the size of the tumor (4), in

humans the relations between weight loss and the size, extent, stage, and type of tumor are less clear (5).

When cancer patients lose weight, they lose both fat and muscle mass (6). In comparison with anorectic patients who have the same degree of weight loss, cancer patients lose relatively more muscle mass (7). Although anorexia is a frequent phenomenon in cancer patients, reduced food intake by itself does not explain these patients' loss of lean body mass. This suggests that derangements in host metabolism must be a major contributing factor (1, 8).

Metabolic alterations in cancer patients include elevated glucose turnover and increased endogenous glucose production, according to previous studies (5, 9-12). Other studies reported net protein catabolism, mainly from muscle, in cancer patients who were losing weight, whereas in cancer patients with stable weights no change in protein catabolism was observed (13). It has been hypothesized that in cancer patients, increased amounts of alanine originating from muscle degradation are used for gluconeogenesis (4, 14), with consequent loss of nitrogen through urea production. However, studies of gluconeogenesis from alanine in tumor-bearing animals (15) and cancer patients (16, 17) have been inconclusive with regard to the relation to weight loss. Furthermore, the studies conducted previously did not account for anorexia as a potential confounder; food intake data were usually not collected.

The incidence of weight loss in lung cancer patients is high, yet anorexia and obstruction are less likely to be the cause of the observed weight loss with this tumor type than with other cancers. Elevated protein turnover rates have been reported in lung cancer patients experiencing weight loss, but the role of gluconeogenesis from protein in the pathogenesis of weight loss in this type of cancer has remained unclear (18).

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The aim of the present study was to evaluate protein-derived gluconeogenesis in a well-defined group of lung cancer patients. Alanine was selected as a precursor of gluconeogenesis because it is the key protein-derived glucose precursor used by the liver (19). Patients who were and who were not losing weight were included to differentiate between possible effects of lung cancer and of the degree of weight loss on gluconeogenesis.

## SUBJECTS AND METHODS

### Subjects

Patients with histologically proven non-small-cell lung cancer who received care at the outpatient department of the University Hospital Rotterdam in the Netherlands were recruited. Two groups of lung cancer patients were studied: those who had lost  $\geq 5\%$  of their body weight in the previous 6 mo (weight-losing, WL) and those who had not lost weight in the previous 6 mo (weight-stable, WS). Patients were only included after an interval of  $\geq 3$  mo after surgery or  $\geq 4$  wk after chemotherapy or radiotherapy. Patients who were in remission or were apparently cured were excluded. Other exclusion criteria were pregnancy, metabolic disease, corticosteroid treatment, liver metastases confirmed by computed tomography or ultrasound, alcohol consumption of  $> 10$  units/wk, and use of a slimming diet. Healthy, weight-stable control subjects were included as a reference group. The study was approved by the Medical Ethical Committee of the Erasmus University Medical Center Rotterdam. All participants signed informed consent forms.

### Dietary intake

Food intake was recorded by using a standard food diary during the 7 d preceding the experiment. The subjects received oral and written information from a trained nutritionist (SL) about the procedures for filling in the diary and measuring all drinks and foods with household measures. Subjects were asked to maintain their usual dietary habits, and none of the subjects reported any significant changes in their diets. Subjects abstained from alcohol for 5 d before the experiment. All medications taken by the subjects were noted.

Household measures were converted into weights by using a standard food conversion table (Department of Human Nutrition, Wageningen, Netherlands) and the nutrition software program KOMMET (version 2.0; B-Ware, Arnhem, Netherlands). The subjects' average daily intakes of energy, fat, carbohydrate, and protein were calculated.

### Study design

We measured the subjects' body weight, height, upper arm circumference, and 4 skinfold thicknesses (triceps, biceps, subscapular, and suprailiac). Skinfold thicknesses were measured with a standard skinfold caliper (Holtain Ltd, London). Body mass index in  $\text{kg}/\text{m}^2$  was calculated, and upper arm muscle circumference was calculated as described by Frisancho (20).

Subjects were studied in the morning after an overnight fast. A cannula ( $0.8 \times 25$  mm) was positioned in the left cubital vein for the infusion of stable isotope tracers. In the contralateral cubital vein, an identical cannula was positioned for blood sampling. To study gluconeogenesis, we prepared a solution containing  $[6,6\text{-}^2\text{H}_2]\text{-D-glucose}$ , 98 atom%, and  $[3\text{-}^{13}\text{C}]\text{-L-alanine}$ , 99 atom% (Mass Trace, Woburn, MA), in water; the solution was sterilized by

autoclaving in glass vials. A priming dose of 0.03 mmol  $[6,6\text{-}^2\text{H}_2]\text{-D-glucose}/\text{kg}$  was administered followed by a continuous infusion of 0.01 mmol  $[6,6\text{-}^2\text{H}_2]\text{-D-glucose} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  for 90 min. Simultaneously, a priming dose of 0.08 mmol  $[3\text{-}^{13}\text{C}]\text{-L-alanine}/\text{kg}$  was given followed by a continuous infusion of 0.04 mmol  $[3\text{-}^{13}\text{C}]\text{-L-alanine} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  over 90 min. Both tracer solutions were infused by using calibrated syringe pumps (Perfusor fm; Braun, Kronberg, Germany).

Venous blood samples were drawn immediately before the isotope infusions were started and then at 10-min intervals from 60 to 90 min, when steady state conditions for the tracer infusions had been achieved. Plasma concentrations of glucose, alanine, insulin, glucagon, and thyroid hormones and isotopic enrichments of glucose and alanine were determined.

### Analytic methods

Blood samples were collected in tubes containing lithium heparin (Vacutainer; Becton Dickinson, Meylan Cedex, France) and were immediately stored on ice. After centrifugation ( $1200 \times g$  for 10 min at  $4^\circ\text{C}$ ) of the samples, the plasma was collected and stored at  $-20^\circ\text{C}$  until analyzed. An aliquot of the infusate was analyzed to document the actual concentrations of the tracers in each study.

Blood glucose concentrations were determined enzymatically with a glucose oxidase and peroxidase assay system (Boehringer Mannheim, Mannheim, Germany). Plasma alanine concentrations were measured enzymatically as described by Williamson (21). Plasma concentrations of insulin and glucagon were determined by radioimmunoassay techniques (Biosource, Fleurus, Belgium and Euro-Diagnostica, Malmo, Sweden, respectively). Serum total thyroxine ( $T_4$ ) and triiodothyronine ( $T_3$ ) concentrations were measured by radioimmunoassay and total reverse triiodothyronine ( $rT_3$ ) concentrations were measured according to the method of Bauer et al (22).

Isotopic enrichments were determined by using the following procedures. Plasma was deproteinized by adding 0.3 mol barium hydroxide/L (Sigma Diagnostics, St Louis) and 0.3 mol zinc sulfate/L (Merck, Darmstadt, Germany). After centrifugation ( $15000 \times g$  for 8 min at  $4^\circ\text{C}$ ), the supernate was applied to an ion-exchange column (mixed bed: AG50W-X8 and AG1-X8, 200–400 mesh, 0.2 g each; BioRad, Hercules, CA). Glucose and alanine were eluted from the column by using water and 4 mol ammonium hydroxide/L (Merck), respectively, and were dried under nitrogen.

A glucose derivative (aldonitril pentaacetate) was made according to the method of Varma et al (23). An alanine *tert*-butyldimethylsilyl derivative was prepared as described by Chaves Das Neves and Vasconcelos (24).

Isotopic enrichments were measured by injecting 1- $\mu\text{L}$  samples with a split ratio of 50:1 on a fused silica capillary column ( $25 \text{ m} \times 0.22 \text{ mm}$ ) coated with 0.11- $\mu\text{m}$  high-temperature 5% phenylpolycarborene siloxane (SGE, Victoria, Australia). The relative isotopic enrichments of deuterated glucose and  $[^{13}\text{C}]\text{alanine}$  were determined by using a Carlo Erba GC8000 gas chromatograph coupled to a Fisons MD800 mass spectrometer (Interscience BV, Breda, Netherlands) in electron impact ionization mode. The CV for enrichment was 0.2 mol% for both  $[6,6\text{-}^2\text{H}_2]\text{glucose}$  and  $[3\text{-}^{13}\text{C}]\text{alanine}$  and no concentration effect was observed at this level of enrichment. Ions were selectively monitored at a mass-to-charge ratio ( $m/z$ ) of 187 for natural glucose and  $m/z$  189 for the deuterated molecule. The isotopic



enrichment of [3-<sup>13</sup>C]alanine was determined at *m/z* 260 and 261 for [1<sup>2</sup>C]alanine and [1<sup>3</sup>C]alanine, respectively (25).

The total enrichment of [1<sup>3</sup>C]glucose was measured separately (aldonitril pentaacetate derivation) by using a gas chromatograph combustion isotope ratio mass spectrometer (Optima; Micro-mass UK, Middlewich, Cheshire, United Kingdom). The [1<sup>3</sup>C]glucose enrichment in atom percent excess (APE) was monitored after combustion to carbon dioxide at mass 44 for carbon-12 and mass 45 for carbon-13.

### Calculations and statistics

Whole-body rate of appearance (Ra) for glucose and alanine was calculated during steady state based on a one-compartment model by using the following formula:

$$Ra = F \times [(IE_i/IE_{ecf}) - 1] \quad (1)$$

where *F* is the isotope infusion rate in mmol·kg<sup>-1</sup>·h<sup>-1</sup>, IE<sub>*i*</sub> is the isotopic enrichment of the infusate in mol percent excess (MPE), and IE<sub>ecf</sub> is the isotopic enrichment of the extracellular fluid (plasma) in MPE (26).

The percentage glucose produced from alanine equals

$$IE [^{13}C]glucose_{plasma} / (IE [^{13}C]alanine_{plasma} \times 0.33) \quad (2)$$

where IE [1<sup>3</sup>C]glucose<sub>plasma</sub> is the isotopic enrichment in plasma (expressed as <sup>13</sup>CO<sub>2</sub> APE). Because alanine has 3 carbon atoms, 100% enrichment of [3-<sup>13</sup>C]alanine in MPE is equivalent to 33% enrichment of <sup>13</sup>CO<sub>2</sub> from alanine (APE). Therefore, the enrichment of alanine (MPE) is multiplied by 0.33 to obtain comparable enrichments (ie, expressed as <sup>13</sup>CO<sub>2</sub> APE) for both alanine and glucose. Gluconeogenesis from alanine in mmol·kg<sup>-1</sup>·h<sup>-1</sup> was then obtained as follows:

$$\text{Percentage glucose from alanine} \times Ra ([^2H_2]glucose) \quad (3)$$

Finally, the percentage of alanine converted into glucose was calculated by dividing the rate of gluconeogenesis from alanine by the rate of appearance of alanine (27).

The results are presented as means ± SEMs. Differences among group means were compared by using analysis of variance with dummies for lung cancer and weight loss as covariates,

with adjustment for age. Bonferroni correction was applied to allow for multiple testing. Correlations between variables were determined by using Pearson's product-moment correlation coefficients. Multiple linear regression analysis was used to analyze interrelationships, with adjustment for possible confounders. Differences were considered statistically significant at *P* < 0.05. Data were analyzed with SPSS for WINDOWS (version 8; SPSS Inc, Chicago).

## RESULTS

### Study population

The subjects were 19 lung cancer patients and 15 healthy control subjects (3 men and 12 women). Of the lung cancer patients, 9 (7 men and 2 women) were classified as WL and 10 (6 men and 4 women) were classified as WS. The cancer patients had been diagnosed with non-small-cell lung cancer at the following stages (World Health Organization grading system): IIIA (1 WS, 3 WL), IIIB (3 WS, 2 WL), and IV (6 WS, 4 WL). The characteristics of the study population are presented in **Table 1**. The cancer patients were significantly older than the control subjects. The WL patients had lost 9.0 ± 1.4 kg or 12% (range: 6–22%) of their pre-illness stable body weight within the 6 mo preceding the study. Body weight, percentage of ideal body weight, body mass index, mid-upper arm circumference, and the sum of 4 skinfold thicknesses were all significantly lower in the WL patients than in the WS patients and the control subjects. Upper arm muscle circumference was slightly, but not significantly, lower in the WL patients than in the WS patients and control subjects. Albumin and transthyretin (pre-albumin) concentrations were lower in the WL patients than in the WS patients and control subjects (*P* < 0.01).

### Dietary intake

None of the subjects reported any changes in their food intake compared with their pre-illness food intake. Carbohydrate intake preceding the experiment was ≥200 g/d in all subjects (**Table 2**). No significant differences in energy, carbohydrate, protein, or fat intakes were found among the groups.

**TABLE 1**

Characteristics of the study population<sup>1</sup>

	Control subjects ( <i>n</i> = 15)	Lung cancer patients	
		WS ( <i>n</i> = 10)	WL ( <i>n</i> = 9)
Age (y)	52 (29–72) <sup>a,b,2</sup>	63 (38–75) <sup>a</sup>	69 (53–81) <sup>b</sup>
Height (m)	1.74 ± 0.02	1.69 ± 0.03	1.71 ± 0.03
Weight (kg)	77.2 ± 2.5 <sup>a</sup>	73.5 ± 3.8 <sup>b</sup>	60.9 ± 3.5 <sup>a,b</sup>
Percentage ideal body weight (%)	123.0 ± 4.1 <sup>a</sup>	119.3 ± 6.4 <sup>b</sup>	96.0 ± 5.0 <sup>a,b</sup>
Weight loss in past 6 mo (%)	—	−2.0 ± 1.0 <sup>a</sup>	12 ± 2 <sup>a</sup>
Body mass index (kg/m <sup>2</sup> )	25.7 ± 0.8 <sup>a</sup>	25.6 ± 1.3 <sup>b</sup>	20.9 ± 1.1 <sup>a,b</sup>
Arm circumference (cm)	32.1 ± 1.0 <sup>a</sup>	30.3 ± 1.4 <sup>b</sup>	26.1 ± 1.2 <sup>a,b</sup>
Arm muscle circumference (cm)	26.1 ± 0.9	26.0 ± 0.9	24.4 ± 1.4
Sum of skinfold thicknesses (mm) <sup>3</sup>	69.6 ± 4.0 <sup>a</sup>	64.6 ± 8.4 <sup>b</sup>	29.8 ± 3.8 <sup>a,b</sup>
Albumin (g/L)	46 ± 1 <sup>a</sup>	44 ± 1 <sup>b,4</sup>	38 ± 2 <sup>a,b,4</sup>
Transthyretin (g/L)	0.29 ± 0.01 <sup>a</sup>	0.28 ± 0.02 <sup>b,4</sup>	0.17 ± 0.02 <sup>a,b</sup>

<sup>1</sup> $\bar{x}$  ± SEM. Differences among group means were determined by using analysis of variance; means within a row with a superscript letter in common are significantly different, *P* < 0.05. WS, weight stable; WL, weight losing.

<sup>2</sup>Range in parentheses.

<sup>3</sup>Calculated as the sum of the biceps, triceps, subscapular, and suprailiac skinfold thicknesses.

<sup>4</sup>One value was missing.

**TABLE 2**Daily intake of energy and selected nutrients in healthy control subjects and weight-stable (WS) and weight-losing (WL) lung cancer patients<sup>1</sup>

	Control subjects (n = 15)	Lung cancer patients	
		WS (n = 10)	WL (n = 9)
Energy (kJ/d)	7838 ± 401	8260 ± 405	8170 ± 644
Fat			
(g/d)	77 ± 5	80 ± 5	74 ± 9
(% of energy)	37 ± 1	37 ± 1	34 ± 2
Carbohydrate			
(g/d)	206 ± 13	224 ± 12	231 ± 16
(% of energy)	45 ± 2	46 ± 1	48 ± 1
Protein			
(g/d)	79 ± 4	85 ± 5	81 ± 5
(% of energy)	17 ± 1	18 ± 1	17 ± 1

<sup>1</sup> $\bar{x} \pm \text{SEM}$ . Data were obtained from 7-d food records. Differences among group means were determined by using analysis of variance, adjusted for age; no significant differences were found.

### Glucose and alanine metabolism

Baseline plasma glucose and alanine concentrations did not differ significantly between the lung cancer patients and healthy control subjects, but glucose concentrations were significantly higher in WL patients than in WS patients (Table 3). Steady state was reached within 60 min after the isotope tracer infusions began, as judged from visual inspection of isotope enrichment profiles. Relative isotopic enrichments of [<sup>2</sup>H<sub>2</sub>]glucose, [<sup>13</sup>C]alanine, and [<sup>13</sup>C]glucose in plasma during steady state did not differ significantly among the groups.

Turnover rates of glucose and alanine are shown in Figure 1. The whole-body glucose turnover rate was significantly higher in WL lung cancer patients than in WS lung cancer patients or healthy control subjects. Similarly, the alanine turnover rate was significantly higher in WL patients than in the other 2 groups. Gluconeogenesis from alanine was significantly elevated in WL patients when compared with both WS patients and control subjects. The percentage of glucose derived from alanine was 64.6 ± 4.2% in WL patients, 60.2 ± 11.8% in WS patients, and 55.0 ± 4.8% in control subjects (NS) and the percentage of alanine converted into glucose was 84.2 ± 6.9%, 70.4 ± 7.1%, and 70.0 ± 7.1% in these 3 groups, respectively (NS). Glucose and alanine turnover rates and gluconeogenesis from alanine were positively correlated with the degree of weight loss ( $r = 0.45$ ,  $P < 0.01$  for all) and inversely correlated with percentage of ideal body weight ( $r = -0.38$ ,  $-0.44$ , and  $-0.50$ , respectively,  $P < 0.05$  for all). The relation between gluconeogenesis from alanine and weight change in lung cancer patients is shown in Figure 2.

No correlations were found between turnover measurements and subject sex or age. Multivariate analyses showed that only the degree of weight loss was a significant predictor of glucose and alanine turnover and of gluconeogenesis from alanine. No associations were found between energy, carbohydrate, and protein intakes on the day before the experiment and plasma glucose or alanine concentrations or turnover measurements.

### Hormone concentrations

Fasting insulin and glucagon concentrations and the insulin-to-glucagon ratio were not significantly different between lung cancer patients and healthy control subjects (Table 4). However,

glucagon concentrations in WL patients were lower than those in WS patients ( $P < 0.01$ ). The insulin-to-glucagon ratio was significantly correlated with alanine turnover ( $r = -0.36$ ,  $P = 0.04$ ). No other significant correlations between insulin or glucagon concentrations and turnover measurements were observed. T<sub>4</sub> and T<sub>3</sub> concentrations did not differ significantly among the groups. In contrast, the rT<sub>3</sub> concentration and rT<sub>3</sub>-to-T<sub>3</sub> ratio were significantly elevated in WL lung cancer patients and were correlated with the degree of weight loss ( $r = 0.47$  and  $0.53$ , respectively,  $P < 0.01$  for both). Reverse T<sub>3</sub> concentrations were also correlated with glucose turnover ( $r = 0.36$ ,  $P = 0.03$ ).

### DISCUSSION

The present study was designed to quantify gluconeogenesis from alanine in both WL and WS patients with advanced lung cancer, as compared with healthy control subjects. Although several authors reported abnormal glucose metabolism in patients with advanced malignant disease (7, 9, 10, 12, 28), few studies have been done with lung cancer patients. Heber et al (11) reported elevated glucose turnover rates in 65% of patients who had non-small-cell lung cancer with weight loss.

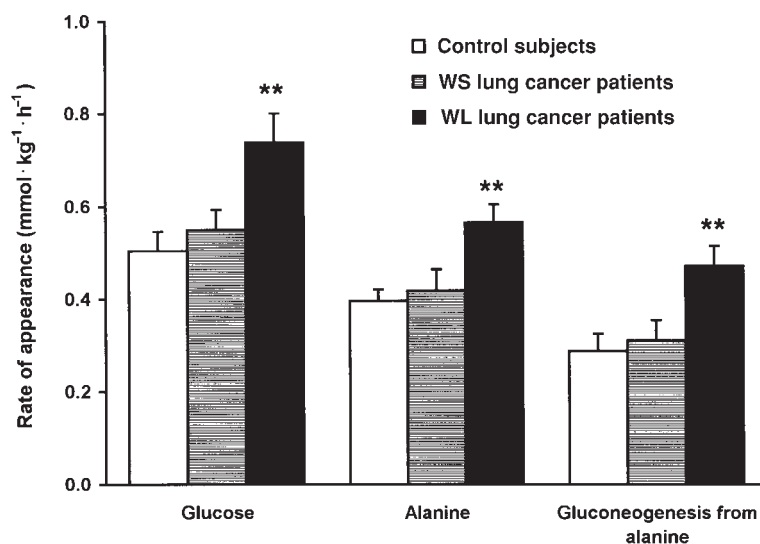
Furthermore, elevated rates of whole-body protein turnover have been measured in cancer patients by using different amino acids (8, 10, 13, 28, 29), but data from lung cancer patients are sparse and inconclusive regarding the role of weight loss. Richards et al (30), who used a primed-constant infusion of [<sup>15</sup>N]glycine, reported increased protein turnover rates in patients who had advanced lung cancer with weight loss. Melville et al (18) also found elevated protein turnover by using [<sup>13</sup>C]leucine in patients with newly diagnosed nonmetastatic lung cancer, but these authors did not observe any relation with weight loss. Fearon et al (31) studied lung cancer patients (stages II or III) who were and who were not losing weight; whole-body protein turnover rates as measured by [<sup>15</sup>N]glycine were elevated in all cancer patients but again, no relation with weight loss was observed. Heber et al (32) used [<sup>14</sup>C]lysine to measure protein turnover rate in lung cancer patients and found that it was elevated and inversely correlated

**TABLE 3**Plasma glucose and alanine concentrations and isotopic enrichments of plasma glucose and alanine during turnover measurements in healthy control subjects and weight-stable (WS) and weight-losing (WL) lung cancer patients<sup>1</sup>

	Control subjects (n = 15)	Lung cancer patients	
		WS (n = 10)	WL (n = 9)
Glucose (mmol/L)	5.9 ± 0.2	5.2 ± 0.2 <sup>a</sup>	5.8 ± 0.3 <sup>a</sup>
Alanine (μmol/L)	347 ± 6	368 ± 8	349 ± 13
Isotopic enrichment of plasma			
[ <sup>2</sup> H <sub>2</sub> ]Glucose (MPE)	2.19 ± 0.25	1.88 ± 0.19	1.55 ± 0.13
[ <sup>13</sup> C]Alanine (MPE)	8.78 ± 0.78	8.32 ± 0.62	7.22 ± 0.60
[ <sup>13</sup> C]Glucose (APE)	1.52 ± 0.14	1.49 ± 0.18	1.53 ± 0.13

<sup>1</sup> $\bar{x} \pm \text{SEM}$ . Isotopic enrichments were determined under steady state conditions during primed-constant infusions of [6,6-<sup>2</sup>H<sub>2</sub>]-D-glucose and [3-<sup>13</sup>C]-L-alanine. Differences among group means were determined by using analysis of variance, adjusted for age; means within a row with a superscript letter in common are significantly different,  $P < 0.05$ . MPE, mole percent excess; APE, <sup>13</sup>CO<sub>2</sub> atom percent excess.





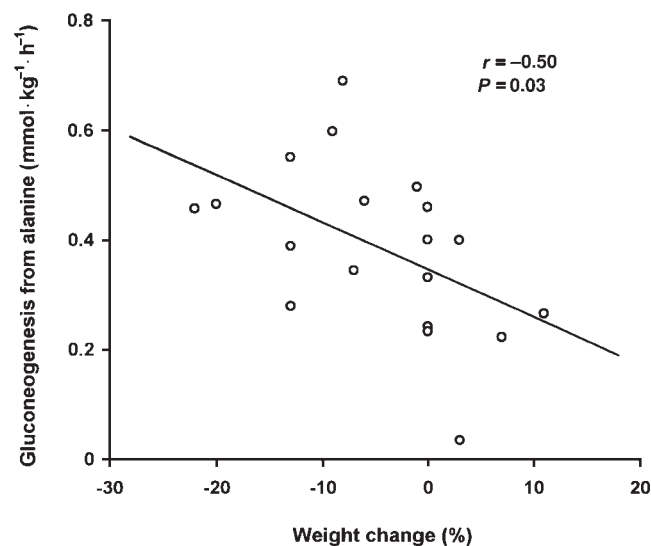
**FIGURE 1.** Mean ( $\pm$ SEM) whole-body rate of appearance (turnover) of glucose and alanine and gluconeogenesis from alanine in healthy control subjects ( $n = 15$ ) and weight-stable (WS,  $n = 10$ ) and weight-losing (WL,  $n = 9$ ) lung cancer patients. Turnover rates were assessed by using primed-constant infusions of  $[6,6\text{-}^2\text{H}_2]\text{glucose}$  and  $[3\text{-}^{13}\text{C}]\text{alanine}$ . \*\*Significantly different from WS lung cancer patients and healthy control subjects,  $P < 0.01$  (ANOVA, adjusted for age).

with percentage of ideal body weight but not with degree of weight loss. In the present study, alanine turnover was significantly correlated with both degree of weight loss and percentage of ideal body weight in lung cancer patients.

To date, gluconeogenesis from alanine in cancer patients has been studied only by using a single bolus infusion of  $[^{14}\text{C}]\text{alanine}$  (ie, not in steady state conditions). Waterhouse et al (16) reported elevated rates of alanine-to-glucose conversion in WL cancer patients with mixed tumor types and metastatic disease when compared with undernourished subjects without cancer. Dietary intake was not reported in that study. Burt et al (17) reported elevated rates of alanine turnover that were not related to the degree of weight loss in patients with localized esophageal cancer. The present study, which was performed under steady state conditions with infusion of  $[^2\text{H}_2]\text{glucose}$  and  $[^{13}\text{C}]\text{alanine}$ , showed that gluconeogenesis from alanine was elevated in WL but not in WS lung cancer patients.

Note that the approach of using  $[^{13}\text{C}]\text{alanine}$  underestimates gluconeogenesis because  $^{13}\text{C}$  is diluted in intracellular pyruvate pools and at oxaloacetate, caused by exchange with the tricarboxylic acid cycle (26). We assumed that the yield of transfer of  $^{13}\text{C}$  from alanine to glucose via the oxaloacetate crossover in the liver and the extent of  $^{13}\text{CO}_2$  reincorporation via gluconeogenesis into carbons 3 and 4 of glucose were identical in all 3 groups. Furthermore, as a result of rapid exchange of  $^{13}\text{C}$  between alanine and pyruvate/lactate, gluconeogenesis may include a contribution from Cori cycling. We were able to measure  $^{13}\text{C}$  enrichment of lactate and found no significant differences among healthy control subjects, WS patients, and WL patients. Food intake was carefully monitored in all subjects by means of dietary records kept for the 7 d preceding the turnover measurements. The results revealed no significant differences in energy intake among the groups; in fact, lung cancer patients tended to have higher energy intakes than did healthy control subjects. This indicates that weight loss and turnover measurements in our study population of lung cancer patients were not related to reduced food intake.

Several mechanisms may be responsible for the elevated gluconeogenesis from alanine in WL lung cancer patients. Increased gluconeogenesis could be caused by decreased insulin or increased glucagon concentrations (33, 34). However, in our study and studies by others (7, 11, 32), fasting insulin concentrations were not decreased in WS or WL lung cancer patients. Furthermore, no significant difference in glucagon concentrations between lung cancer patients and healthy control subjects was observed. Rofe et al (34) reported an altered insulin response and insulin-to-glucagon ratio in cancer patients with various tumor types,



**FIGURE 2.** Gluconeogenesis from alanine in lung cancer patients plotted against the percentage change from pre-illness stable body weight during the previous 6 mo ( $n = 19$ ;  $r$  is Pearson's product-moment correlation coefficient). Gluconeogenesis from alanine was assessed by using primed-constant infusions of  $[6,6\text{-}^2\text{H}_2]\text{glucose}$  and  $[3\text{-}^{13}\text{C}]\text{alanine}$ .

**TABLE 4**

Fasting hormone concentrations in healthy control subjects and weight-stable (WS) and weight-losing (WL) lung cancer patients<sup>1</sup>

	Control subjects (n = 15)	Lung cancer patients	
		WS (n = 10)	WL (n = 9)
Insulin (pmol/L)	64.2 ± 8.5	77.3 ± 19.9	57.4 ± 15.4
Glucagon (pmol/L)	5.7 ± 0.7	8.1 ± 0.9	6.5 ± 1.6
Insulin:glucagon	12.4 ± 1.6	9.4 ± 1.4	18.4 ± 7.3
T <sub>4</sub> (nmol/L)	100 ± 10	107 ± 5	103 ± 8
T <sub>3</sub> (nmol/L)	1.85 ± 0.12	1.87 ± 0.06	1.63 ± 0.17
rT <sub>3</sub> (nmol/L)	0.26 ± 0.02 <sup>a</sup>	0.30 ± 0.03 <sup>b</sup>	0.40 ± 0.05 <sup>a,b</sup>
rT <sub>3</sub> :T <sub>3</sub>	0.15 ± 0.02 <sup>a</sup>	0.16 ± 0.02	0.27 ± 0.05 <sup>a</sup>

<sup>1</sup> $\bar{x} \pm \text{SEM}$ . Differences among group means were determined by using analysis of variance, adjusted for age; means within a row with a superscript letter in common are significantly different,  $P < 0.05$ . T<sub>4</sub>, thyroxine; T<sub>3</sub>, triiodothyronine; rT<sub>3</sub>, reverse triiodothyronine.

<sup>2</sup>One value was missing.


although there was no consistent relation with the degree of weight loss in these patients. Clearly, it is not possible to define mechanisms on the basis of plasma concentrations of insulin or glucagon alone; however, our data do not support a predominant role for insulin or glucagon concentrations in causing the observed alterations in gluconeogenesis in WL lung cancer patients.

A second hypothesis is that elevated gluconeogenesis from alanine is the result of increased substrate availability or increased transport of alanine into the liver. In animal studies *in vivo*, alanine concentrations in the livers of tumor-bearing hosts were elevated (35) but plasma concentrations were normal (36) or decreased (33), which suggests increased uptake of alanine by the liver. Reduced plasma alanine concentrations in WL lung cancer patients were reported by some authors (11, 37). However, in the present study we did not detect differences in plasma alanine concentrations among WL and WS lung cancer patients and healthy control subjects despite a 36% increase in alanine flux in the WL cancer patients. Moreover, no correlation between plasma alanine concentrations and alanine turnover rates was observed ( $r = -0.08$ ,  $P = 0.67$ ). Based on our results, it would therefore seem unlikely that the elevated alanine turnover rate would result simply from increased plasma alanine concentrations. However, the possibility of increased alanine uptake by the liver cannot be ruled out on the basis of our data.

Other possible mechanisms are the acute-phase response and circulating cytokines (38–40) or elevated activity of one or more gluconeogenic enzymes within the liver, as has been shown *in vitro* for phosphoenolpyruvate carboxykinase, glucose-6-phosphatase (41), and pyruvate carboxylase (42). In WL lung cancer patients, hydrazine sulfate reduced glucose production (43) and increased plasma alanine concentrations (44), probably by inhibiting phosphoenolpyruvate carboxykinase. However, no data on hepatic gluconeogenic enzyme activities in humans with lung cancer are available.

Gluconeogenesis is an energy-consuming process, and enhanced conversion of alanine into glucose could therefore act as an energy-depleting mechanism. Although elevated gluconeogenesis may be an important factor in the pathology of weight loss in lung cancer, this does not preclude contributions from other energy-wasting processes to weight loss in lung cancer patients. Nevertheless, increased gluconeogenesis from alanine could result in loss of

body protein. It is thought that loss of muscle mass contributes to impaired muscle function and decreased survival (2, 45).

In summary, elevated rates of glucose and alanine turnover and gluconeogenesis from alanine were detected in patients who had advanced lung cancer with weight loss. These metabolic abnormalities were not related to either the presence of lung cancer or reduced energy intake. Prospective studies on the relation between elevated gluconeogenesis from alanine and weight loss in lung cancer, and the underlying mechanisms, are warranted. 

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