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Inter-relationships Between Single Carbon Units' Metabolism and Resting Energy Expenditure in Weight-losing Patients with Small Cell Lung Cancer. Effects of Methionine Supply and Chemotherapy

H. Sengeløv, O.P. Hansen, L. Simonsen, J. Bülow, O.J. Nielsen and L. Ovesen

The one-carbon unit metabolism was investigated in 8 weight-losing patients with small cell carcinoma of the lung (SCLC). At diagnosis, 6 of the 8 patients had elevated formiminoglutamic acid (FIGLU) excretion after a histidine load, suggesting a lack of one-carbon units. In accordance, a significant decrease of FIGLU excretion was observed in the patients after oral administration of DL-methionine for 4 days. The elevated FIGLU excretion was positively correlated to weight loss prior to diagnosis and negatively correlated to serum albumin at time of diagnosis. After 3 months of combination chemotherapy, FIGLU excretion was reduced in all patients except 1, who had progressive disease. Despite the elevated FIGLU excretions, all patients had normal blood folate levels. The resting energy expenditure (REE) was recorded in 7 patients, and a significant, postive correlation was observed between pretreatment FIGLU excretion and REE, although the REE measured in this group of patients was within the normal range. These data demonstrate an increased demand of "active" one-carbon units in energy consumption in a group of weight-losing cancer patients. The one-carbon unit deficit was reconditioned by oral administration of the one-carbon unit donor DL-methionine.

Key words: one-carbon units, energy expenditure, FIGLU excretion, metabolism, methionine, small cell lung cancer

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INTRODUCTION

TO MAINTAIN a stationary body weight, a delicate equilibrium between caloric intake and total energy expenditure must exist. Obviously, this balance deteriorates in cancer cachexia, but the physiological derailments causing the weight loss had been a matter of much debate. Clinical and experimental observations in cancer cachexia have documented both qualitative and quantitative metabolic disturbances. Among these are: (1) elevated resting energy expenditure (REE) [1, 2], (2) diet-induced hyperthermogenesis [3, 4] and (3) changes in the intermediary metabolism of carbohydrates, proteins and lipids [5–11].

Transfer of carbon-1-units involving tetrahydrofolic acid or S-adenosylmethionine plays a crucial role in *de novo* synthesis of basic cell constituents (Figure 1). The conversion of the hydroxyamino acid serine to glycine is quantitatively the most important donor of "active" single carbon units in the human

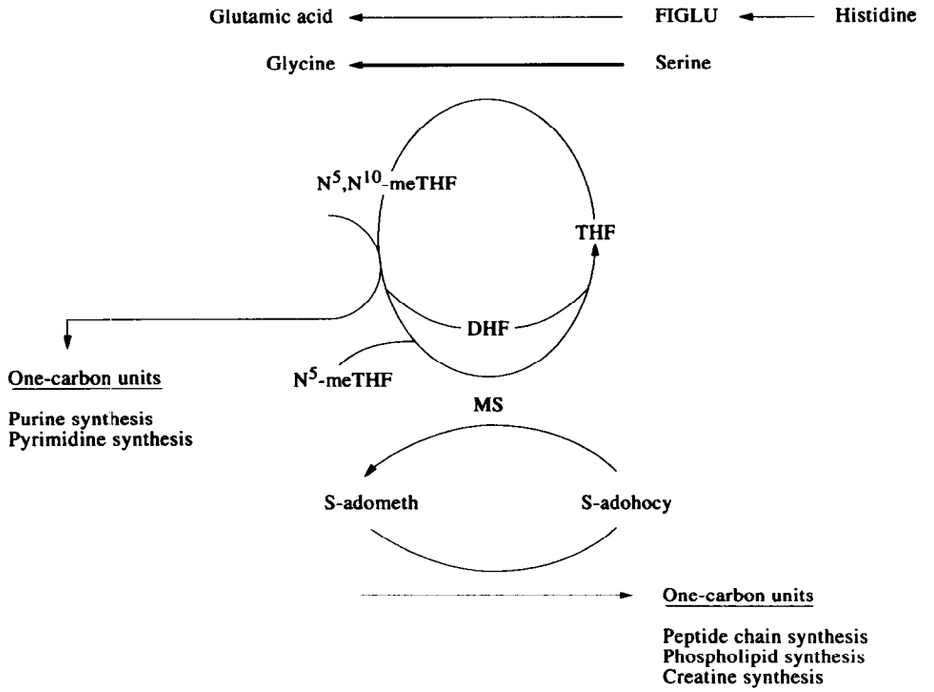

Figure 1. Metabolic inter-relationships in *de novo* synthesis of carbon-1-units and histidine degradation. The metabolic pathways utilising obligate carbon-1-unit transfer from either carbon-1-folate monoglutamate derivatives or S-adenosylmethionine in formation of essential cell constituents are shown. THF, tetrahydrofolic acid; N^5-meTHF, N^5-methyl-THF; N^5,N^{10}-meTHF, N^5,N^{10}-methylene-THF; S-adometh, S-adenosylmethionine; S-adohocy, S-adenosylhomocysteine; MS, methionine synthetase; DHF, dihydrofolate.

metabolism. The intermetabolic pathway comprises reduced tetrahydrofolate (THF) as an obligate precursor for the synthesis of reduced carbon-1-folate monoglutamate derivatives that are essential for cell replication [12]. The intermetabolic methionine derivative S-adenosylmethionine initiates the formation of precursor peptides in the synthesis of proteins, and is an obligate carbon-1-unit donor in the synthesis of creatine and phospholipids. Degradation of the amino acid histidine to glutamate and reduced carbon-1-folate derivatives by an enzyme complex in the liver also requires the presence of THF. In many patients with neoplasms, the histidine degradation is partly blocked at this level. Hansen and colleagues [13] have demonstrated releasing effects of methyl-cobalamin and methionine that indicate insufficient reactivation of methionine synthetase to overcome the metabolic demand for carbon-1-units in these patients.

The purpose of the present study was to evaluate the relative deficiency of carbon-1 units in small cell lung cancer (SCLC) patients by the urinary excretion of formiminoglutamic acid (FIGLU) following a histidine load.

PATIENTS AND METHODS

8 patients (7 males, 1 female, age 62–71 years) with histologically verified SCLC were examined twice, before and at least 3 weeks after the last combination chemotherapy, given every fourth week for 3–6 months. The chemotherapeutic regimen comprised combinations of the following drugs: teniposide, cisplatin, vincristine and doxorubicin according to running protocols. The study was approved by the Ethics Commitee for Medical Research in Copenhagen and all patients gave informed consent.

Whole body resting energy expenditure (REE)

All subjects were examined after an overnight fast, and height, body weight and lean body mass (LBM) were measured at each occasion. LBM was measured by the bioelectrical impedance method [14]. REE was measured by the open circuit ventilated hood system for 1 h, oxygen with an electrochemical oxygen sensor (Amtec Thermox, Pittsburg, Pennsylvannia, U.S.A.), and carbon dioxide with an infrared carbon dioxide sensor (Amtec Thermox). The airflow through the system, the relative air humidity and the air temperature were measured, and all parameters were online monitored on a computer. Details of the system and calibration have been described elsewhere [15]. Energy expenditure (EE) was calculated using the formula $EE = [(15.48 + 5.55*RQ)VO_2]$ kJ/min [16]. REE was measured at diagnosis and after 3 months of chemotherapeutic treatment. The metabolic data presented in this study has been published previously [4].

Formiminoglutamic acid (FIGLU) excretion was measured in close association to the metabolic evaluation, but the two procedures were always conducted on separate days. The first FIGLU excretion was measured at diagnosis and 3 months later. Fifteen grams of L-histidine were given orally to the patient after an overnight fast. Urine passed was discarded within the first half hour but then collected for the next 7½ hours. Urine

Correspondence to O.P. Hansen.
H. Sengelov, O.P. Hansen and L. Ovesen are at the Department of Medicine P; L. Simonsen and J. Bülow are at the Department of Clinical Physiology, Bispebjerg Hospital, Bispebjerg Bakke 23, DK-2400 Copenhagen; and O.J. Nielsen is at the Department of Nephrology, State University Hospital, Rigshospitalet, Copenhagen, Denmark.
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volume was registered, and the FIGLU content was measured as previously described [17]. Ten grammes of DL-methionine were given orally once a day during the following 4 days. The FIGLU excretion after a histidine load was repeated on day 6. The urine samples were added to 10 ml 1 M HCl/l urine and stored at −20°C until FIGLU determination.

Blood analysis

Blood folates, serum cobalamin, serum albumin (S-albumin), serum lactate dehydrogenase (S-LDH) and blood haemoglobin (Hgb) concentrations were analysed by standard laboratory methods. Serum erythropoietin (S-Epo) was measured as described by Nielsen [18].

Statistical analysis

Data are given as median values with ranges. For analysis of paired data, the Wilcoxon one-sample rank sum test was used and two-tailed P value calculated. Rank correlation was calculated using the Spearman test, and expressed as two-tailed significance of t corrected for ties. P values ≤5% were considered significant.

RESULTS

7 patients had a partial remission, i.e. >50% shrinkage of the tumour mass, estimated by X-ray examination after 3–6 months treatment with combination chemotherapy. One patient (no. 6) had tumour progression, i.e. >25% increase in tumour mass at the time of re-examination. The patients had a lean body mass (LBM) of 55.1 + 6.6 kg (mean ± 2 S.D.) before chemotherapy, hardly different from their pretreatment LBM (56.1 ± 11.4 kg).

The FIGLU excretions before start and after termination of chemotherapy are shown in Figures 2 and 3, respectively. Prior to chemotherapeutic treatment, a significant (P = 0.03) reduction in FIGLU excretion was seen following the DL-methionine load (Figure 2). After 3 months of chemotherapy, FIGLU excretion was reduced compared to the pretreatment value in all patients except 1, who had progressive disease. 4 of 5 patients further decreased their FIGLU excretion after DL-methionine load (Figure 3). The initial FIGLU excretion before start of chemotherapy was positively correlated with REE (r = 0.77, P = 0.05) and weight loss prior to diagnosis (r = 0.89, P = 0.07). No relation between FIGLU excretion and S-LDH was found.

REE was 86.8 (range 67.4–100.3) J/kg LBM/min, and had not changed after chemotherapy. A close relationship (r = 0.71, P = 0.07) between REE and S-LDH was found before the start of chemotherapy (Figure 4).

S-Epo increased in all patients after chemotherapy (P = 0.02, Table 1). Before chemotherapy 4 of 8 patients had normal Hgb values, the other 4 had slightly reduced Hgb values without increased S-Epo. S-albumin did not change in the 6 patients who had pre- and postchemotherapy values measured (Table 1).

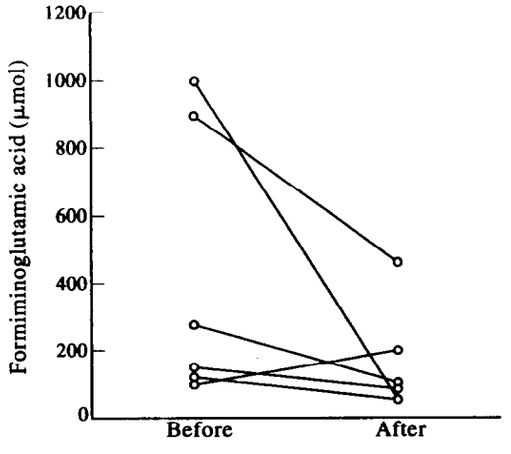

Figure 2. Formiminoglutamic acid (FIGLU) excretion (7½ h) following histidine load evaluated before and after 10 g DL-methionine/day for 4 days. Measurements were performed prior to chemotherapy. A pair of data were extremely high, and are not included in the figure: 4420 μmol FIGLU before DL-methionine and 2531 μmol after. A significant decrease in FIGLU excretion was observed, P = 0.03. n = 6 before, n = 6 after.

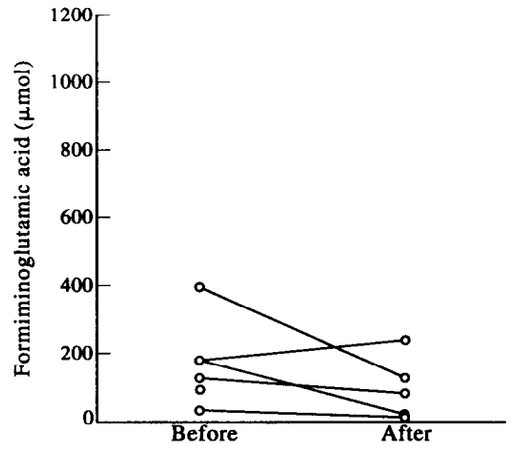

Figure 3. FIGLU excretion (7½ h) following histidine load evaluated before and after 10 g DL-methionine/day for 4 days. Measurements were performed after 3 months of chemotherapy. No significant decrease in FIGLU excretion is observed after DL-methionine. n = 6 before, n = 5 after.

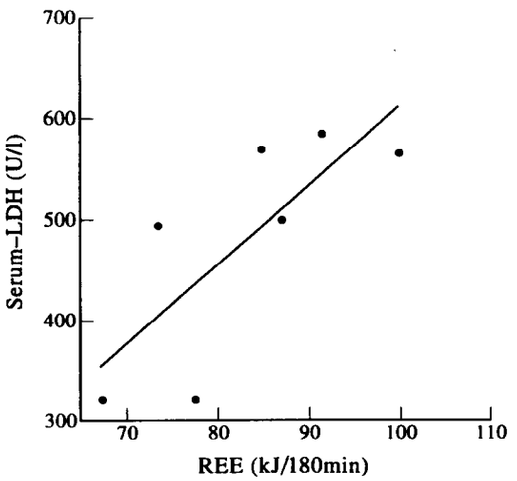

Figure 4. Correlation between resting energy expenditure (REE) and serum lactate dehydrogenase (LDH) recorded before chemotherapy was initiated. Regression line is indicated. Spearmans r = 0.71, two-tailed P = 0.07.

Table 1. Blood analyses before and 3 months after chemotherapeutic treatment

	Hgb (mmol/l)		S-Epo (mU/ml)		S-albumin (μmol/l)		S-LDH (U/l)	S-folates (mmol/l)	
	Before	After	Before	After	Before	After	Before	Before	After
Normal range	♀:7.4–9.6 ♂:8.4–10.8		18–58		♀:550–760 ♂:600–810		150–500	>0.2	
Patient no.									
1	8.5	7.5	17.1	164.0	621	562	493	0.7	0.7
2	8.3	6.9	13.3	51.3	517	632	583	0.2	0.2
3	7.4	6.2	20.0	105.0	556	494	321	0.6	0.4
4	6.9	*	30.8	*	424	*	564	0.2	*
5	6.7	6.9	19.8	41.5	452	—	320	0.7	0.3
6	9.8	6.6	20.6	70.0	656	640	562	0.3	0.7
7	8.1	7.3	16.3	52.0	421	—	498	0.6	0.5
8	7.5	7.2	17.8	30.4	422	371	—	0.8	0.5

* Died before analysis.

Pretreatment S-albumin was negatively correlated to pretreatment FIGLU excretion ($r = 0.79, P = 0.05$). However, weight loss before and after chemotherapy did not correlate to S-albumin. Blood folate and S-cobalamin did not change after treatment with chemotherapy, and consequently no relation to FIGLU excretion was found.

DISCUSSION

Based on the initially increased FIGLU excretion, which is significantly reduced by a methionine-supplemented diet, we suggest that our patients have an insufficient reactivation of methionine synthetase to meet additional metabolic demand for one-carbon compounds in intermediary liver metabolism. Sufficient amounts of carbon-1-units for synthesis are mainly delivered either as THF carbon-1-derivatives, or as S-adenosyl-methionine. Shortage of reduced folate carbon-1-compounds substantially increases the stress on the alternative pathway that includes carbon-1-units supply from methionine [19, 20].

The correlation between pretreatment REE and FIGLU excretion indicates that even in untreated tumour-bearing humans, the greater part of carbon-1-unit metabolism in normal liver cell metabolism is used for energy consumption. Serum LDH is a major indicator of tumour cell mass and prognosis in patients with SCLC [21]. The lack of a significant relationship between S-LDH and FIGLU excretion does not suggest a quantitatively proportional substitution of carbon-1-units from liver cell metabolism owing to the proposed tumour cell methionine dependency [22,23]. Alternatively, the REE is closely related to S-LDH in patients with SCLC indicating that a substantial part of the resting energy demand is related to actual size of the growing tumour cell mass. Thus, this relationship might be one causal factor in the correlation between weight loss and prognosis as demonstrated in patients with SCLC [24]. Hence, other metabolic energy-consuming processes must be inversely reduced with tumour burden in patients with SCLC as the REE in our 8 patients does not differ significantly from that in healthy individuals [4].

Among the biochemical variables that separate weight-losing cancer patients from weight-stable cancer patients, are S-albumin and Hgb [3]. The intermetabolic methionine derivative, S-adenosyl-methionine, initiates the formation of precursor peptides in the synthesis of proteins such as enzymes, carrier proteins (albumin), hormones (insulin, triiodthyronine), and haematopoietic growth factors (erythropoietin) [25, 26]. Subnormal S-albumin is an important sign of malnutrition, but a late indicator of protein deficiency. Our finding of a significant correlation between S-albumin and FIGLU excretion suggests the presence of protracted metabolic disturbances in the protein synthesis associated with altered carbon-1-metabolism in patients with SCLC. The previously demonstrated rapid disappearance of methionine in plasma, and the reduced plasma levels of methionine, serine and glycine in patients with solid tumours [27, 28], are in accordance with our results indicating a shortage of methionine supply for the synthesis of precursor peptides. We, therefore, assume that the demonstrated, inadequate low to subnormal values of S-Epo in relation to Hgb in untreated patients (Table 1) are probably due to defective Epo synthesis. After 3–6 months of chemotherapy, the situation changed, showing an adequate Epo response, but without adequate red cell production, resulting in subnormal Hgb.

In contrast to patients with myelomatosis and normal FIGLU excretion, where the methionine load increases the FIGLU excretion [13], we also observed a decreased FIGLU excretion after DL-methionine diet supplementation in patients with tumour regression. This suggests a continuously increased demand for carbon-1-units, which is met sufficiently in local liver metabolism. Thus, after 3–6 months of chemotherapy, the patients' carbon-1-unit intermediary metabolism is seemingly changed, but not normalised. Accordingly, we assume that the decrease in demand for carbon-1-units in responding patients is related to a lower energy demand caused by a reduction in growing tumour cell mass. Moreover, these metabolic changes are followed by a steady LBM. Rebuilding of normal tissue in responding patients with SCLC demands high supplements of extra energy intake in combination with "active" carbon-1-units and essential amino acids. Although supporting the demand for carbon-1-units, a few days oral treatment with supplementary methionine is obviously too short to induce an anabolic metabolic state. In addition, when glutamine depletion is present in muscle cells, even parenteral high-calorie nutrition becomes valueless [11], unless L-glutamine is also given intravenously [29]. Postsurgery catecholamine-induced tissue changes in the amino acid pattern, principally a fall in glutamine content [29], might also

be present in SCLC patients, according to plasma analysis of amino acids in these patients [28]. Thus, in chemotherapy-treated patients with SCLC, the inability to gain a sufficient rise in body weight within 5 months after the start of chemotherapy (unpublished data) could be caused by a simultaneous depletion of S-adenosyl-methionine in liver tissue and L-glutamine depletion in muscle tissue. Further studies are needed to confirm this suggestion, including trials with supplementation of glutamine and methionine in excess. However, an inherent risk exists in that methionine and glutamine supply may stimulate tumour growth [30].

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