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Serum Amino Acids in Weight-losing Patients with Cancer and Tuberculosis

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Abstract—*A study of arterial and arterio-venous amino acid concentration differences across the forearm was performed in 19 weight-losing cancer (CWL) patients (9 with lung cancer and 10 with other types of cancer), 8 weight-losing patients with active pulmonary tuberculosis (TWL) and 10 normal controls. Arterial concentrations of many of the amino acids measured were found to be lower in CWL than in TWL patients. In addition, the data suggested a venous excess of amino acids in the CWL patients compared with TWL patients and controls. The increased release of alanine from forearm muscles in the CWL group, together with the low arterial glycogenic amino acid levels, supports the concept of enhanced gluconeogenesis in CWL patients. Low arterial amino acid levels and possible increased release of amino acids from forearm muscle in CWL patients implies enhanced proteolysis with increased central clearance or tumour sequestration of these amino acids, though decreased proteogenesis cannot be excluded in accounting for the venous excesses in this group. Hypocitrullinemia in lung cancer patients was marked, and possible mechanisms to account for this are discussed.*

INTRODUCTION

THERE are numerous potential abnormalities in protein metabolism in the weight-losing cancer patient (CWL). Accelerated protein synthesis and sequestration of certain amino acids by the tumour coupled with impaired muscle proteogenesis and enhanced muscle proteolysis in these patients [1, 2] could predictably produce a severe imbalance in protein metabolic homeostasis.

One of the difficulties in studying this problem is to ascertain which changes are unique to cancer patients, so we have undertaken to investigate serum amino acid levels in weight-losing cancer patients and in patients with a non-malignant chronic debilitating disease associated with weight loss. For the latter group we have elected to study weight-losing patients with tuberculosis (TWL).

We have measured the arterial and venous amino acids across the forearm in CWL, TWL and normal control patients to establish whether any significant disturbance in muscle proteolysis or proteogenesis exists. Increased muscle proteolysis is characterised by a venous excess of amino acids and elevated arterial levels of essential branched-chain amino acids [3]. Conversely, decreased proteogenesis, as may occur in protein-calorie malnutrition, is characterised by decreased arterio-venous amino acid differences and reduced arterial plasma levels of essential branched-chain amino acids [3–5].

MATERIALS AND METHODS

Approval for this study was obtained from the Hospital Ethical Committee and informed consent was obtained from all patients studied.

Patients with various solid tumours (excluding those of the gastrointestinal tract), who had lost at least 10% body weight during the preceding 6 weeks, had received no radiotherapy, surgery or chemotherapy during this time and had no evidence of intestinal obstruction or dysphagia, were selected for study. All patients had locally advanced disease and there was no clinical evidence of metastatic disease, with the exception of one patient with adenocarcinoma of the lung

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who had a cerebral metastasis. Nine patients had lung cancer (three adenocarcinoma, three oat-cell, two squamous-cell and one large-cell carcinoma), nine patients had stage III carcinoma of the cervix and one patient had stage III carcinoma of the ovary. Data derived from cancer patients were analysed together and then separated into lung and non-lung cancer groups to overcome the possible bias in the former group resulting from the many paraneoplastic endocrine-mediated metabolic alterations associated with lung cancer.

In addition, patients with active pulmonary tuberculosis who had lost at least 10% body weight during the previous 6 weeks were selected, as well as 'normal' patients who had been admitted to hospital for minor surgical procedures such as hernia repair, haemorrhoidectomy and varicose vein stripping. All control patients were studied pre-operatively and none gave a history of recent weight loss.

None of the patients studied had diabetes or other endocrine abnormalities, nor was there biochemical evidence of hepatic or renal dysfunction in any of them.

Blood samples were collected after a 12-hr overnight fast. Patients in all three groups were ambulatory in a hospital environment and blood specimens were obtained early in the morning before any physical exertion. Venous blood was collected from a large antecubital vein without stasis and arterial blood was collected by radial artery puncture from the same arm. The clotted blood was centrifuged immediately and the serum was deproteinised by treatment with sulphosalicylic acid and centrifugation before storage at -20°C until analysis. Amino acids were measured by ion-exchange chromatography using lithium buffers on a Model 121-M Beckman Amino Acid Analyser. The experimental error was found to be less than 5%.

Statistical evaluation of data

Comparisons of arterial amino acids and arterio-venous differences were evaluated by analysis of variance using square root transformations where variances were not homogeneous.

RESULTS

There were 19 CWL patients (9 males, 10 females; average age 49 ± 11 yr), 8 TWL patients (5 males, 3 females; average age 43 ± 12 yr) and 10 normal controls (6 males, 3 females; average age 46 ± 14 yr).

Arterial amino acids

Mean values for arterial amino acid levels are shown in Table 1.

(a) Branched-chain amino acids. All branched-chain amino acids were present at significantly lower concentrations in the arterial blood of CWL patients than in TWL patients. Although the mean values for branched-chain amino acids were lower in the CWL patients than in normal controls, these differences were not statistically significant.

(b) Glycine. One of the hallmarks of chronic malnutrition is an elevation of plasma glycine. While glycine levels in TWL patients did not differ from controls, values were lower in the CWL patients than in TWL patients ($P < 0.005$) and controls ($P < 0.05$).

(c) Citrulline. Citrulline concentrations were significantly decreased in the CWL patients when compared with control patients (18.7 ± 10.1 vs 28.3 ± 12, $P < 0.05$). This difference was brought about by low levels in the lung cancer patients (15.4 ± 6.1), there being no difference between CWL patients without lung cancer and controls.

While citrulline levels were lower in lung cancer patients compared with TWL patients (15.4 ± 6.1 vs 24.1 ± 11.4), this difference was not statistically significant.

(d) Other amino acids. Alanine, serine, aspartate, ornithine, phenylalanine and tyrosine concentrations were all significantly lower in CWL patients than in TWL patients. In almost every case this difference was brought about largely by raised levels in the TWL patients when compared with controls.

Although arterial levels of most of these amino acids were decreased in CWL patients when compared with controls, these differences did not achieve significant levels.

Histidine concentrations were significantly lower in TWL patients than in controls (43.7 ± 4.9 vs 55.4 ± 12.3, $P < 0.05$) but not CWL patients.

Arterio-venous differences of amino acids

The means of arterio-venous differences for individual amino acids in the three groups of patients are shown in parentheses in Table 1. Qualitative assessment of the data showed a significantly increased release of leucine and alanine from forearm muscles in CWL patients ($P = 0.05$) which was not noted in TWL patients or controls. Although there appeared to be other amino acids with increased venous excess in CWL patients, the small numbers of patients studied may have affected significance levels. The data were also assessed quantitatively by counting the number of patients in each group with 10 or more amino acids in venous excess. The groups were compared in a 2×2 contingency table and a simple Chi-square test applied.

Table 1. Arterial amino acid levels and arterio-venous differences

	Cancer (μmol/l)	Tuberculosis (μmol/l)	Control (μmol/l)
Essential amino acids			
Valine	145.0 ± 41.1 (0) †$P < 0.05$	181.0 ± 30.1 (+7.2)	165.0 ± 38.3 (+8.6)
Leucine	85.0 ± 26.5 (-5.7) †$P < 0.05$	115.9 ± 19.5 (+0.6)	97.5 ± 27.1 (+3.6)
Isoleucine	41.4 ± 10.7 (-1.9) †$P < 0.01$	53.4 ± 8.2 (+2.3)	43.8 ± 10.3 (+1.7)
Threonine	82.3 ± 26.3 (-5.4) †$P < 0.06$	98.0 ± 14.4 (-7.0)	93.3 ± 39.6 (-1.9)
Methionine	14.3 ± 4.4 (+0.2)	16.2 ± 2.5 (+0.1)	16.3 ± 3.9 (0)
Lysine	122.1 ± 32.4 (-9.2)	129.6 ± 17.0 (-4.1)	126.5 ± 26.6 (-4.9)
Phenylalanine	54.4 ± 18.4 (-4.4) †$P < 0.05$	72.0 ± 17.1 (-2.5)	50.3 ± 19.7 (+0.8)
Non-essential amino acids			
Serine	107.7 ± 27.8 (+6.2) †$P < 0.005$	158.1 ± 40.0 (+3.8)	131.4 ± 39.4 (+7.4)
Alanine	218.7 ± 67.3 (-35.8) †$P < 0.05$	294.2 ± 51.6 (-23.3)	227.9 ± 74.1 (-18.3)
Proline	129.9 ± 40.5 (-5.1)	154.8 ± 14.8 (-9.1)	140.9 ± 51.0 (+4.2)
Citrulline	18.7 ± 10.1 (+0.4) *$P < 0.05$	24.1 ± 11.4 (+3.2)	28.3 ± 12.0 (+2.4)
Glycine	182.9 ± 41.3 (-11.1) †$P < 0.005$ *$P < 0.05$	240.1 ± 33.2 (+2.1)	219.2 ± 42.6 (+10.8)
½-Cystine	84.9 ± 32.4 (-0.5)	71.1 ± 11.6 (-1.0)	69.3 ± 12.1 (0)
Tyrosine	39.3 ± 9.8 (-3.3) †$P < 0.05$	49.0 ± 8.7 (+1.0)	39.7 ± 9.8 (-0.4)
Ornithine	89.4 ± 53.7 (-5.2) †$P < 0.001$	167.1 ± 22.8 (-1.2) *$P < 0.0001$	72.5 ± 43.1 (+1.2)
Histidine	47.5 ± 10.9 (+3.7)	43.7 ± 4.9 (-0.3) *$P < 0.05$	55.4 ± 12.3 (-1.6)
Arginine	82.3 ± 24.2 (-0.8)	100.6 ± 17.8 (-7.4)	85.1 ± 25.5 (-0.7)
Glutamine and glutamic acid	382.8 ± 49.3 (-14.0)	432.6 ± 40.3 (-29.0)	405.0 ± 30.3 (+38.7)
Asparagine and aspartic acid	97.1 ± 32.6 (-7.8) †$P < 0.001$	149.9 ± 45.9 (+10.8) *$P < 0.05$	110.2 ± 22.8 (+3.8)

Arterio-venous differences are given in parentheses. For a-v differences a minus sign denotes venous excess and a plus sign denotes arterial excess (see text for P values of a-v differences).
*Significant difference compared with control group.
†Significant difference compared with TB group (TWL).

Although there were more CWL patients with ⩾10 amino acids in venous excess (55%) than TWL patients (25%) or normal controls (20%), these differences failed to achieve statistical significance ($0.05 < P < 0.1$).

Independent analysis of lung cancer and non-lung cancer patients

With the notable exception of hypocitrullinemia in the lung cancer patients, there were no other differences in the amino acid profiles between the cancer groups.

Both groups, therefore, displayed decreased arterial amino acid levels and a tendency towards increased amino acid release from forearm muscles when compared with TWL or control patients.

DISCUSSION

We chose to compare the CWL patients with TWL patients so that neither group was biased regarding excessive hypophagia or intestinal malfunction secondary to gastrointestinal surgery or obstruction, all of which could produce distinctive metabolic changes in their own right. The comparison between the amino acid profiles of CWL and TWL patients demonstrated several differences, some of which have not been previously documented.

The striking feature of this study was the finding that there were significantly lower arterial levels of 12 out of 19 amino acids in the CWL patients when compared with TWL patients. There are two possible explanations to account for this:

(1) Increased clearance of these amino acids by liver, kidney and other central tissues, as has been found to occur in septic and post-trauma patients [6]. This would also concur with the concept that the tumour-bearing host is less able to utilise lean tissue-conserving mechanisms than the non-tumour-bearing host [7].

Special mention should be made of the glycogenic amino acids alanine and serine, both of which were reduced in CWL patients. It is generally assumed that low arterial concentrations of glycogenic amino acids reflect an increased hepatic uptake of these amino acids for gluconeogenesis. Increased gluconeogenesis has been well described in weight-losing cancer patients [8, 9]. Furthermore, the increased release of alanine from forearm muscle in CWL patients is consistent with enhanced gluconeogenesis in these patients and concurs with the findings of Clarke *et al.* [10]. High levels of glycogenic amino acids in TWL patients may reflect an attempt to conserve protein by limiting commitment of these amino acids to gluconeogenesis.

(2) Increased sequestration of amino acids by the tumour, which may have an absolute requirement for those amino acids it cannot synthesise, has already been described for glutamine [11] and asparagine [12].

Glycine concentrations in CWL and TWL patients were not elevated significantly above those of control patients and this is consistent with our impression that these patients did not suffer from chronic malnutrition.

The significant hypocitrullinemia in weight-losing lung cancer patients may have been due to altered handling of citrulline by the liver or alterations in urea synthesis, possibly resulting from a tumour-mediated effect on hepatic polyamine synthesis [13], which would decrease the ornithine substrate for citrulline synthesis.

When measuring arterio-venous differences ideally muscle blood flow should be determined, as differences in flow may theoretically affect the uptake and loss of amino acids in a muscle group. Furthermore, it would have been more desirable to measure arterial–femoral vein differences to allow for study of a larger muscle mass than forearm measurements allow. We were unable to make such measurements for a variety of practical and ethical reasons. However, we endeavoured to ensure that patient conditions and ambient surroundings were as constant as possible for each sampling episode.

Probably as a result of the above considerations, it was difficult to show conclusively whether there was an overall net venous excess of amino acids in the CWL patients. Nevertheless, 53% of CWL patients had an excessive release of amino acids from forearm muscle compared with 25% of TWL and 20% of controls and, furthermore, there was a significant venous excess of leucine and alanine in CWL patients.

It is interesting that Clowes *et al.* [6] showed a faster release of amino acids from skeletal muscle in septic and post-trauma patients, and this would again represent a striking similarity with the CWL patients if the venous excess was on the basis of enhanced proteolysis.

The finding that there was a significant venous excess of leucine in the CWL patients does not really help to differentiate between enhanced proteolysis or decreased proteogenesis as the underlying cause for this. During enhanced muscle proteolysis resulting from starvation there is known to be an increase in the intracellular pool size of branched-chain amino acids which is associated with release of these amino acids, leading to elevated arterial levels [14]. Likewise, the under-utilisation of these amino acids by skeletal muscle might also lead to an increased venous excess. If there is an under-utilisation of amino acids in CWL patients it is unlikely to be wholly on the basis of an underlying protein-calorie malnutrition, as in the latter situation there is a decreased venous excess of amino acids, probably as a result of decreased proteogenesis to facilitate protein sparing [15]. Furthermore, low arterial glycine concentrations in these patients support the contention that malnutrition did not play a major role in these amino acid changes (*vide supra*).

Under-utilisation of amino acids might result from decreased proteogenesis, which has been demonstrated in skeletal muscle of cancer patients [1, 2]. Alternatively, preferential sequestration of certain amino acids by the tumour may result in an incomplete complement of amino acids presented to skeletal muscle, thereby reducing its capacity for proteogenesis.

CONCLUSIONS

An assessment of arterial and venous amino acids across the forearm in wasting cancer patients compared with wasting patients with tuberculosis and normal controls revealed the following:

1. Arterial levels of a large number of amino acids were significantly lower in CWL patients than in TWL patients. This may have been due to increased central clearance of these amino acids in CWL patients and may reflect a decreased capacity to conserve body protein. Alternatively, this could be explained at least partly on the basis of enhanced tumour sequestration of certain amino acids.

2. Decreased arterial glycogenic amino acids in CWL patients coupled with an enhanced venous excess of alanine from forearm muscle is suggestive evidence for enhanced gluconeogenesis in these patients.
3. Evidence for an increased venous excess of amino acids in CWL patients is suggestive but not compelling. This may be due to increased proteolysis or under-utilisation of amino acids associated with decreased muscle proteogenesis in this group.
4. Weight-losing cancer patients with lung cancer had low arterial citrulline concentrations, which may be due to an overall increased central clearance of amino acids or to alterations in urea cycle activity related to hepatic polyamine synthesis.

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