Longitudinal Changes of Biochemical Parameters in Muscle During Critical Illness

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The study was undertaken to characterize the time course of biochemical parameters in skeletal muscle during critical illness to gain information for the design of a suitable protocol for interventional studies using metabolic or nutritional manipulation. Critically ill patients in our intensive care unit ([ICU] N = 9) were investigated on two separate sampling occasions with percutaneous muscle biopsies for determination of protein, nucleic acids, free amino acids, energy-rich phosphates, fat, water, and electrolytes. The first biopsy specimen was taken 3 to 11 days after admission and the second biopsy specimen 3 to 7 days later. Protein concentration, expressed as alkali-soluble protein (ASP)/DNA, decreased by 12% ($P < .02$) between the two biopsies. The total free amino acid content was only 50% of normal, but remained unaltered over time. In particular, the concentration of glutamine remained low, approximately 25% of normal. In contrast, branched-chain amino acid (BCAA) increased by 25% ($P < .05$) and phenylalanine by 55% ($P < .05$) between biopsies. The fat content related to fat-free solid (FFS) increased by 130% ($P < .001$) between the two biopsies. Muscle water did not change during the study period. The extracellular portion was double the normal value when related to FFS. Intracellular water, on the other hand, was outside the 95% confidence interval for normal values in the second biopsy. The concentrations of adenosine triphosphate (ATP), creatine, phosphocreatine, and the phosphorylated fraction of total creatine remained at the same level between the two biopsies. We conclude that in critically ill patients, there is a decrease in protein content over time and increases in BCAA, phenylalanine, and fat content, while the low glutamine level and high extracellular water content remain unaltered. The temporal alterations were well characterized after a 5-day study period.

CRITICALLY ILL PATIENTS who stay for a long time in the intensive care unit (ICU) comprise less than 10% of the total number of patients treated therein, but generate greater than 50% of the costs for intensive care. Nevertheless, studies focusing on the metabolic pathophysiology of these patients are not numerous.

The loss of skeletal muscle proteins and muscle mass during critical illness is a considerable clinical problem. Despite conventional parenteral nutrition, amino acids are exported from the periphery to vital organs in the splanchnic area for gluconeogenesis, oxidation, ureagenesis, and protein synthesis. During critical illness, the ability of muscle to produce substrates for export to other tissues may become a limiting factor. A deteriorated nutritional condition is therefore a negative factor in the prognosis of the disease. In addition, protein depletion in muscle leads to reduced muscle strength, which may affect the length of the hospital stay and recovery period.

The metabolic and nutritional treatment of critically ill patients is aimed at minimizing the negative effects of the altered protein metabolism. Evaluating treatment in terms of parameters related to clinical outcome will necessitate large study groups, implying multicenter studies, to obtain results within a reasonable period. Therefore, pilot studies with biochemical outcome parameters are necessary to make initial evaluations of new therapeutic regimens. Here, a descriptive study of the temporal pattern of biochemical parameters in skeletal muscle provides background material on how to design interventional studies of new nutritional treatments.

Regardless of the underlying disease, critically ill patients show a uniform pattern of biochemical changes with a reduction of the protein in skeletal muscle expressed as alkali-soluble protein (ASP)/DNA, and the protein reduction is correlated with the duration of critical illness. There is a substantial reduction of the skeletal muscle concentration of free glutamine and an increase in the level of branched-chain amino acids (BCAAs).[1-3] Extracellular water is increased and leads to a slightly higher than normal total water content in muscle.

Single observations of ICU patients have been reported in previous study.[4] In the present investigation, the hypothesis is that the time course of these biochemical alterations in the individual patient may be used as a marker for progression of the depletion and therefore be useful when evaluating the effect of metabolic and/or nutritional treatment.

SUBJECTS AND METHODS

Patients

Critically ill patients (N = 9) admitted to the ICU were included in the study (Table 1). They had been subjected to trauma, surgical complications, and/or bacteremia, and the inclusion criteria were coagulation parameters allowing percutaneous muscle biopsies. Patients were studied on two occasions 3 to 7 days apart. The results of biopsy 1 have been presented elsewhere.[4]

Clinical characterization was made using the APACHE II score, which was 11 to 30 on admission to the ICU. The first biopsy was performed 3 to 11 days after admission with APACHE II scores of 7 to 17, and at the second biopsy 3 to 7 days later, APACHE II scores were 5 to 25. The general condition of four patients improved during the study period, which is reflected by the changes in APACHE II mean scores of 21 (admission), 14.8 (first biopsy), and 11.8 (second biopsy). The general condition deteriorated in five patients between the two biopsies; the changes in the APACHE II mean score were 18.6 (admission), 11.6 (first biopsy), and 14.4 (second biopsy). APACHE scoring was applied

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Table 1. Subject Characteristics

Diagnosis	Between Biopsies	Previous Health Condition	Patient No.	Age (yr)	Sex	Body Weight (kg)	APACHE II			Biopsy 1 on Day of Critical Illness (n)	ICU Treatment (d)	Nonsurvivors	
							Admission	Biopsy 1	Biopsy 2			ICU	6 Mo Later
1. Appendicitis. Ileus.	Recovery, transient renal insufficiency	Diabetes mellitus	1	69	M	115	20	17	15	11	22		
2. Aortic aneurysm, large postsurgical bleeding. Sepsis.	Recovery, improved oxygenation on ventilator	Hypertension	2	73	M	94	19	15	12	5	29		
3. Intestinal resection. Peritonitis. Re-operation.	Increasing septic symptoms, peritoneal abscess, re-operated day after second biopsy	Asthma, chronic bronchitis; alcohol abuse; smoker; heart infarction 3 times	3	63	M	82	22	15	11	8	41		
4. Bilateral pneumonia.	Recovery, improved oxygenation, off ventilator on day after second biopsy	Diabetes mellitus; schizophrenia	4	44	M	58	24	10	5	3	9		
5. Untreated asthma. Circulatory arrest on emergency ward.	Increasing symptoms of brain damage; died 1 day after second biopsy	Healthy	5	38	F	72	30	15	25	4	9	NS	
6. Abdominal shot-wound, intestinal perforation.	Increasing septic symptoms, left lung atelectasis	Healthy	6	32	M	74	14	10	11	3	16	NS	
7. Hemorrhagic pancreatitis. laparotomy.	Unchanged condition, fever	Diabetes mellitus	7	58	M	111	12	11	14	9	39		
8. Esophageal stenosis (malignant tumor). Pneumonia.	Slowly weaning off the ventilator; wound rupture on day of second biopsy	Treated thyrotoxicosis	8	70	M	60	21	17	15	8	29		NS
9. Multiple fractures, subarachnoidal bleeding, flail-chest.	Deteriorated pulmonary function, pneumothorax, atelectasis	Healthy	9	51	F	60	15	7	11	7	27		

according to Knaus et al,[5] and when patients were sedated, no points were scored on the Glasgow Coma Scale.

All patients were mechanically ventilated, and when required, analgesia and sedation was provided with morphine hydrochloride (Morfin; Kabi Pharmacia, Uppsala, Sweden) and benzodiazepines (Dormicum; Hoffman-la Roche, Basel, Switzerland) or propofol (Diprivan; ICI Pharmaceuticals, Macclesfield, England). Prophylaxis of thrombosis was administered to all patients with heparin sodium (Heparin; Kabi Pharmacia) 5,000 U subcutaneously twice daily. All patients except no. 9 were given antibiotics at the time of the investigation, due to culture-verified infection or for infection prophylaxis in association with intestinal surgery. The patients received parenteral nutrition from day 3 in the ICU after establishment of a stable circulation. Parenteral nutrition given during the 24 hours preceding the biopsy supplied 102 kJ/kg body weight/24 h (range, 73 to 128) as isocaloric amounts of fat and glucose (Intralipid 20%; Kabi Pharmacia, Stockholm Sweden) and 0.13 g N/kg body weight/24 h (range, 0.08 to 0.20). Amino acids were supplied as Vamin-Glucose (Kabi-Pharmacia, Stockholm). Fat, amino acids, and glucose were given between 10 AM and 10 PM, and during the night a low-calorie glucose infusion was administered.

At the first sampling occasion, patients had been in the ICU for 3 to 11 days (Table 1). Muscle and blood sampling was performed in the morning when the patients were in a postabsorptive state, ie, only a low-calorie glucose infusion had been administered since 10 PM the previous day. Muscle biopsies were performed for determination of free amino acids, energy-rich phosphates, protein, nucleic acids, fat, water, and electrolytes. Blood samples were taken from an indwelling arterial catheter for determination of amino acid and total protein in plasma.

The patients (or if communication with the patient was not possible, the relatives) were informed of the purpose, procedure, and possible risks involved in the study and in the sampling procedure before providing informed consent to participate. The study protocol was approved by the Ethics Committee of the Karolinska Institute, Stockholm, Sweden.

Analytical Technique

Muscle biopsy. The percutaneous muscle biopsy technique is described in detail elsewhere.[6] In brief, after local anesthesia of the skin with prilocain (Citanest 1%; Astra, Södertälje, Sweden), a muscle biopsy specimen was taken from the lateral portion of the quadriceps femoris muscle approximately 15 cm above the knee, and the second specimen was taken from the other leg. Special care was taken not to introduce any local anesthetic into the muscle tissue, since it would produce an error in the analyses of water and electrolytes. The muscle tissue was dissected carefully and rapidly of visible fat and connective tissue. The biopsy specimen with a wet weight of approximately 150 mg was divided into portions for determination of water and energy-rich phosphates (70 mg) and for amino acid determination (two × 25 mg). Each piece of muscle tissue was weighed three times within 24 seconds on an automatic electrobalance to calculate the wet weight at time zero (Cahn29; Cahn Instruments, Cerritos, CA). Specimens taken for determination of water, electrolytes, protein, nucleic aids, and energy-rich phosphates were frozen in liquid nitrogen 1 to 2 minutes after sampling, and samples taken for amino acid analysis were frozen within 5 minutes after sampling. All samples were stored at −80°C until analysis.

Muscle specimens taken for determination of energy-rich phosphagens were frozen in liquid nitrogen 1 to 2 minutes after sampling, ie, not

immediately, which was the case in the investigations by Liaw et al[7,8] and Bergström et al.[9] Söderlund and Hultman[10] have demonstrated that the phosphocreatine content in muscle after a 1-minute delay of freezing corresponds to the level in fresh muscle. The adenosine triphosphate (ATP) level in muscle is not affected by a delay in freezing as long as 6 minutes.

Amino acid analysis. The muscle samples (20 to 30 mg wet weight) were homogenized in a glass homogenizer with 4% sulfosalicylic acid solution, also containing norleucine as the internal standard, before analysis. Precipitated proteins were sedimented by centrifugation, and free amino acids in the supernatant were separated and quantified by ion-exchange chromatography using an automated amino acid analyzer (Alpha Plus; LKB, Bromma, Sweden) with a DC-6 ion-exchange resin (Durrum, Interaction, CA) and lithium citrate buffers. OPA (*O*-phthaldehyde) derivatives of the amino acids were quantified by fluorescence with excitation 350 nm and emission at 420 nm (RF-535 fluorescence monitor; Shimadzu, Kyoto, Japan).

Determination of water, electrolytes, protein, nucleic acids, and chloride. The assays are described in detail by Forsberg et al.[11] In brief, frozen samples of 60 to 100 mg wet weight were freeze-dried, weighed, fat-extracted with petroleum ether, and reweighed to obtain the total muscle water and fat. The sample was powdered and divided into three portions. Electrolytes were extracted from one portion (2 to 3 mg) with nitric acid. The supernatant was diluted with an ionization and compensation solution ($Fe(NO_3)_3$, H_2SO_4, H_3PO_4, and $CaCO_3$)), and sodium, potassium, and magnesium were determined by an atomic absorption spectrophotometer (751; Instrumentation Laboratory, Wilmington, MA). Chloride was analyzed in the same supernatant with electrometric titration against silver nitrate using a pH meter (pH-M 62; Radiometer, Copenhagen, Denmark).

The second portion of the powder (3 to 4 mg) was precipitated with perchloric acid (PCA), cooled, and centrifuged, the precipitate was washed with PCA and dissolved with KOH, and the solution was analyzed for ASP by the method of Lowry et al.[12] For the assay of RNA, the powder was prepared as for the assay of protein, and after precipitation of protein and DNA with PCA, the supernatants were assayed by the direct spectrophotometric method of Fleck and Begg.[13] For DNA extraction, the precipitate was hydrolyzed with PCA and DNA was estimated by the diphenyl-amine reaction. The extraction procedure is a modified technique described by Munro and Fleck.[14]

The third portion (4 to 5 mg) was extracted with PCA containing EDTA in an ice bath, neutralized with $KHCO_3$, and assayed for energy-rich phosphates (ATP, phosphocreatine, and creatine). Enzymatic reactions were measured on a spectrophotometer. Values for energy-rich phosphates are from six patients, no. 4 to 9, due to failure of the freezer in which these samples from patients no. 1 to 3 were stored.

Calculations

Water distribution in the muscle sample was calculated from the chloride and water content of the muscle sample. Since chloride is freely diffusable across the muscle cell membrane and follows Nernst's equation,[15] the ratio of extracellular and intracellular chloride concentrations will be 26:1, assuming a resting membrane potential of −87.2 mV. The extracellular chloride concentration was calculated from the plasma concentration, which was corrected for protein content and Donnan equilibrium (0.96). Estimates of total, extracellular, and intracellular water content of the sample were based on these analyses and calculated as described by Bergström et al.[16]

Concentrations of the intracellular electrolytes sodium, potassium, and magnesium were calculated by subtracting extracellularly distributed electrolytes from the total muscle content, assuming that interstitial electrolyte concentrations were equal to those in plasma.

Statistics

Values are given as the mean ± SD if not indicated otherwise. Ranges are given within parentheses as indicated. Student's *t* test for paired samples was used to compare first and second biopsy results. Correlations were calculated using Pearson's correlation coefficient.

For comparison, normal levels of the biochemical parameters have been stated as 95% confidence intervals in the tables. The analyses were made with the same method and in the same laboratory used in the present investigation. Normal levels of free amino acids are from Hammarqvist et al,[17] protein, water, fat, and electrolytes from Forsberg et al,[11] and energy-rich phosphates from 12 healthy volunteers (Gamrin L, Berg H, and Hultman E, unpublished data, 1993).

RESULTS

Nine critically ill patients subjected to trauma, surgical complications, and/or bacteremia were investigated on two separate sampling occasions with measurements of skeletal muscle concentrations of protein, nucleic acids, free amino acids, energy-rich phosphates, fat, water, and electrolytes.

Protein and Nucleic Acids

The protein concentration expressed as ASP/DNA decreased by 12% ($P < .02$) between the two biopsies (Fig 1). RNA/DNA, reflecting the capacity for protein synthesis, remained unaltered, while RNA/FFS and RNA/ASP increased by 19% ($P < .01$) and 17% ($P < .01$), respectively, reaching values outside the 95% confidence interval for the reference group (Table 2).

Amino Acids

BCAA increased between biopsy 1 and biopsy 2 by 25% ($P < .05$) (Fig 2). In particular, the increase of valine was 22% ($P < .05$) and of leucine 33% ($P < .05$). Phenylalanine increased by 55% ($P < .05$) (Fig 2). Glutamine remained at the

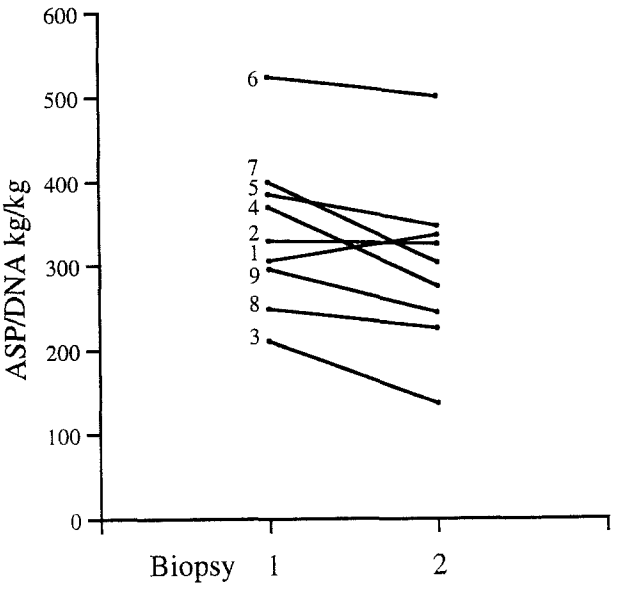

Fig 1. Changes in skeletal muscle protein content in critically ill patients (N = 9) expressed as ASP/DNA on 2 separate biopsy occasions 3 to 7 days apart. Numbers 1 to 9 refer to the individual patients (Table 1).

Table 2. Skeletal Muscle Protein and Nucleic Acids in Critically Ill Patients Investigated With Repeated Sampling

Parameter	Reference Base	Unit	Biopsy 1	Biopsy 2	Healthy Reference Group (n = 21) 95% Confidence Interval
DNA	FFS	g/kg	2.2 ± 0.6	2.3 ± 0.5	1.9-2.3
RNA			3.7 ± 0.6	4.4 ± 1.1†	3.4-3.7
ASP			699 ± 18	702 ± 15	691-709
DNA	ASP	g/kg	3.0 ± 0.9	3.7 ± 1.6	2.8-3.3
RNA			5.4 ± 0.9	6.3 ± 1.6†	4.9-
ASP	DNA	kg/kg	341 ± 93	300 ± 100*	311-370
RNA			1.8 ± 0.4	1.8 ± 0.3	1.4-1.8

NOTE. Data are the mean ± SD. The 95% confidence intervals for a healthy reference group are from Forsberg et al.[11]

*$P < .05$, †$P < .01$: significant difference from paired values obtained at biopsy 1.

same level in the two biopsies. BCAA and aromatic amino acids were above and glutamine and glutamate were below the reference interval (Table 3).

Plasma amino acids were analyzed on both sampling occasions. The levels of plasma amino acids remained unaltered between the two biopsies.

Fat, Water, and Electrolytes

The fat content related to FFS increased by 130% ($P < .001$) between the two biopsies (Fig 3).

Total tissue water and also intracellular and extracellular water did not change between the first and second biopsy. Nevertheless, total tissue water was higher than the 95% confidence interval for normals. Intracellular water, on the other hand, was below this interval at the second biopsy (Table 4).

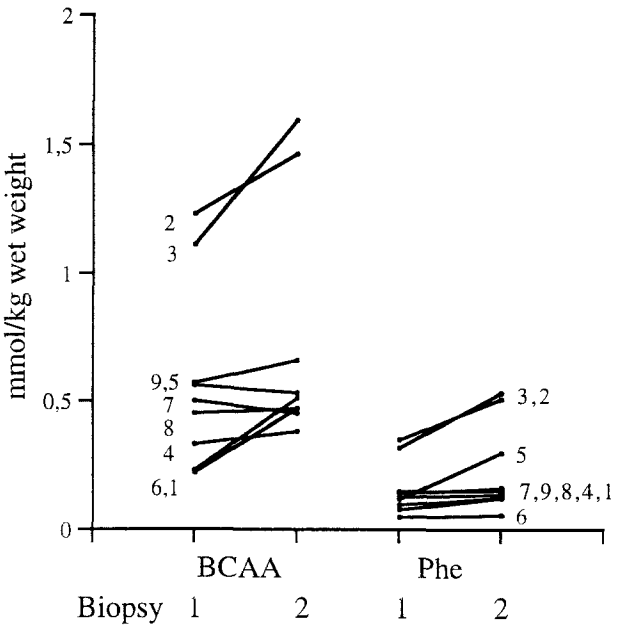

Fig 2. Changes in skeletal muscle BCAA and phenylalanine content in critically ill patients (N = 9) on 2 separate biopsy occasions 3 to 7 days apart. Numbers 1 to 9 refer to the individual patients (Table 1).

There were no changes in tissue electrolyte concentrations. The extracellular constituents sodium and chloride were well above the reference interval when related to the FFS of the muscle tissue.

Energy-Rich Phosphates

Concentrations of ATP, creatine, phosphocreatine, and the phosphorylated fraction of total creatine remained at the same level between the two biopsies. Total creatine and free creatine related to FFS were higher than normal, while the fractions of phosphocreatine from total creatine and ATP related to total creatine were lower than the 95% reference interval. Due to freezer failure, values from patients no. 1 to 3 could not be obtained (Table 5).

DISCUSSION

The patients studied comprise a selection of critically ill patients in the ICU with regard to the severity of illness and the course of the disease. Inclusion criteria for the study were (1) ability to undergo muscle biopsies, and (2) an expected ICU stay of longer than 4 days after the first biopsy to allow a second tissue specimen to be taken. In addition, no patients were included until they were circulatory-stable in general terms. Consequently, all the investigated patients came from the group staying 7 days or longer in the ICU, which is less than 10% of the total number of patients admitted to the ICU. Still, it is this group of patients who are most likely to benefit from careful metabolic and nutritional surveillance with the possibility of improved nutritional treatment in the future.

The continuous decrease in the protein content of skeletal muscle, measured here as ASP/DNA, was the most characteristic and reproducible finding (Fig 1). This biochemical measure parallels the clinically well-established loss of skeletal muscle mass, which is seen in all critically ill patients regardless of the underlying disease and the course of the illness. Low levels of ASP/DNA have been reported,[4,18] but the longitudinal relation to the duration of critical illness in the individual patient has not been emphasized. The mechanism for the loss of muscle proteins still has not been fully elucidated, but a mismatch between protein synthesis and degradation must be at hand. It has been demonstrated that the release of 3-methylhistidine from peripheral tissue is markedly increased in septic patients, indicating a higher than normal rate of protein degradation.[19] On the other hand, muscle protein synthesis is only marginally decreased.[20]

The capacity for protein synthesis is roughly reflected by RNA concentration in the tissue. The apparent increase in the concentration of RNA in relation to FFS or ASP must be regarded primarily as a reflection of the decrease in the reference base, ie, the protein content, and not an actual increase in RNA. RNA concentration is not likely to change rapidly during the course of critical illness, which is in agreement with the finding of an unaltered concentration of RNA in relation to DNA, and also of the only marginally decreased rate of protein synthesis in critical illness.[20] There is a progressive reduction of the RNA concentration over time, since there is a statistical correlation between RNA/DNA and the day of critical illness,[4] but this decrease must be slower than the reduction in protein

Table 3. Free Amino Acids in Skeletal Muscle and Plasma in Critically Ill Patients Investigated With Repeated Sampling

	Muscle (mmol/g wet weight)			Plasma (μmol/L)		
Amino Acid	Biopsy 1	Biopsy 2	Healthy Reference Group (N = 17) 95% Confidence Interval	Biopsy 1	Biopsy 2	Healthy Reference Group (N = 17) 95% Confidence Interval
Taurine	8.81 ± 3.76	10.10 ± 4.39	11.09-14.29	37.1 ± 31.3	47.9 ± 28.1	36-59
Aspartate	0.73 ± 0.33	0.94 ± 0.41	0.85-1.30	9.70 ± 6.10	10.0 ± 5.8	4-6
Threonine	0.48 ± 0.24	0.48 ± 0.22	0.38-0.52	72.7 ± 56.3	60.0 ± 20.1	84-135
Serine	0.53 ± 0.24	0.61 ± 0.29	0.43-0.54	64.5 ± 25.0	65.8 ± 17.8	86-119
Asparagine	0.34 ± 0.13	0.41 ± 0.16	0.29-0.38	36.4 ± 19.5	34.3 ± 13.3	40-49
Glutamate	1.13 ± 0.56	1.59 ± 0.80	2.68-3.67	58.5 ± 28.5	64.5 ± 47.4	37-67
Glutamine	3.38 ± 1.80	3.22 ± 1.32	11.11-13.69	355 ± 127	319 ± 93	422-519
Glycine	1.13 ± 0.40	1.33 ± 0.44	0.89-1.26	134 ± 60	124 ± 35	172-228
Alanine	2.43 ± 1.42	2.48 ± 1.40	1.51-1.93	209 ± 83	174 ± 60	181-266
Valine	0.25 ± 0.15	0.30 ± 0.16*	0.17-0.21	172 ± 64	177 ± 71	140-189
Methionine	0.08 ± 0.05	0.10 ± 0.08	0.03-0.04	26.0 ± 27.4	16.5 ± 4.5	13-21
Isoleucine	0.12 ± 0.07	0.14 ± 0.09	0.05-0.07	39.0 ± 21.1	41.4 ± 18.3	38-50
Leucine	0.21 ± 0.15	0.28 ± 0.21*	0.10-0.13	88.5 ± 34.3	93.3 ± 34.9	78-103
Tyrosine	0.13 ± 0.08	0.14 ± 0.11	0.08-0.11	57.9 ± 17.2	56.9 ± 15.9	36-52
Phenylalanine	0.16 ± 0.10	0.23 ± 0.17*	0.05-0.08	92.7 ± 28.5	127 ± 59.2	34-48
Ornithine	0.10 ± 0.05	0.09 ± 0.04	0.12-0.24	40.8 ± 12.8	48.6 ± 29.5	45-67
Lysine	0.34 ± 0.20	0.35 ± 0.18	0.63-0.84	123 ± 49	106 ± 29.9	115-142
Histidine	0.16 ± 0.11	0.16 ± 0.10	0.22-0.29	53.7 ± 14.7	43.3 ± 6.4	61-74
Carnosine	4.19 ± 1.82	3.99 ± 1.42				
Tryptophan				32.6 ± 20.3	35.0 ± 9.5	29-38
Arginine	0.27 ± 0.30	0.30 ± 0.34	0.40-0.56	45.2 ± 28.0	43.8 ± 14.4	61-74
Total amino acids	12.3 ± 4.58	13.5 ± 3.74	20.85-26.26	1,860 ± 609	1,829 ± 475	1,789-2,195
BCAA	0.58 ± 0.36	0.72 ± 0.46*	0.28-0.40	299 ± 111	312 ± 122	257-284
EAA	2.00 ± 1.04	2.24 ± 1.16	2.38-3.05	741 ± 249	774 ± 225	699-887
AAA	0.29 ± 0.18	0.39 ± 0.29	0.13-0.18	182 ± 60	219 ± 72*	70-100
BAA	0.77 ± 0.47	0.81 ± 0.42	1.25-1.68	216 ± 94	197 ± 41	242-284
Glutamine fraction of total amino acids	0.27 ± 0.08	0.24 ± 0.08	0.47-0.61	0.19 ± 0.03	0.18 ± 0.04	0.22-0.25

NOTE. Data are the mean ± SD. The 95% confidence intervals for a healthy reference group are from Hammarqvist et al.[17]
Abbreviations: EAA, essential amino acids; AAA, aromatic amino acids; BAA, basic amino acids.
*$P < .05$, significant difference from paired values obtained at biopsy 1.

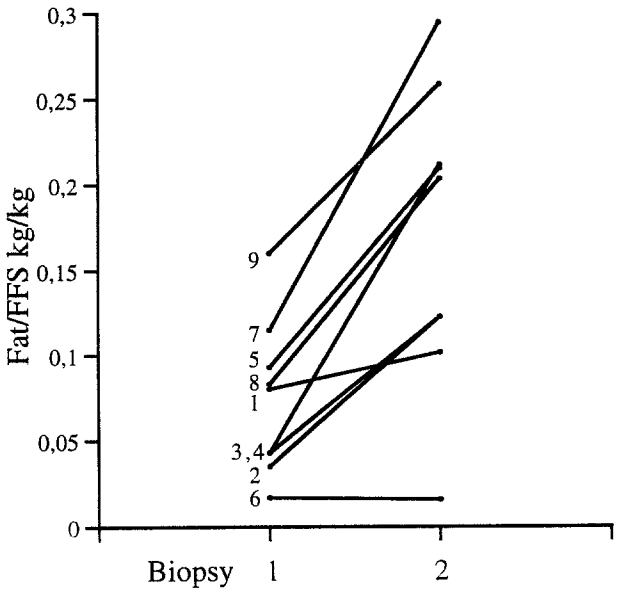

Fig 3. Changes in skeletal muscle fat content in critically ill patients (N = 9) on 2 separate biopsy occasions 3 to 7 days apart. Numbers 1 to 9 refer to the individual patients (Table 1).

Table 4. Skeletal Muscle Fat, Water, and Electrolytes in Critically Ill Patients Investigated With Repeated Sampling

Parameter	Reference Base	Unit	Biopsy 1	Biopsy 2	Healthy Reference Group (N = 21) 95% Confidence Interval
Fat	FFS	kg/kg	0.07 ± 0.05	0.17 ± 0.09*	0.06-0.12
H_2Om		L/kg	3.72 ± 0.54	3.67 ± 0.32	3.30-3.43
H_2Oe			0.92 ± 0.40	1.06 ± 0.24	0.39-0.53
H_2Oi			2.80 ± 0.32	2.33 ± 0.52	2.87-2.94
Cl		mmol/kg	120 ± 40	139 ± 34	57-69
Na			162 ± 41	179 ± 37	83-97
K			431 ± 17	416 ± 14	429-441
Mg			42.6 ± 1.6	41.8 ± 2.1	40.2-42.1
K/Mg			10.1 ± 0.7	10.0 ± 0.5	10.4-10.8
K	ASP	mmol/kg	617 ± 25	594 ± 29	662-644
Mg			61.0 ± 2.5	59.3 ± 2.7	57.6-60.0
K	DNA	mol/kg	208 ± 51	201 ± 55	193-228
Mg			20.8 ± 6.1	20.4 ± 6.3	18.1-21.9

NOTE. Data are the mean ± SD. The 95% confidence intervals for healthy reference group are from Forsberg et al.[11]
Abbreviations: H_2Om, total muscle water; H_2Oe, extracellular water; H_2Oi, intracellular water.
*$P < .01$, significant difference from paired values obtained at biopsy 1.

Table 5. Skeletal Muscle Energy-Rich Phosphates in Critically Ill Patients Investigated With Repeated Sampling

Parameter	Reference Base	Unit	Biopsy 1	Biopsy 2	Healthy Reference Group (N = 12) 95% Confidence Interval
ATP	FFS	mmol/kg	24.1 ± 2.3	21.6 ± 3.7	23.6-25.7
Phospho-creatine			80.5 ± 7.9	72.5 ± 12.5	77.0-85.8
Creatine			69.7 ± 5.5	66.8 ± 9.8	32.0-42.72
Total creatine			150 ± 11	139 ± 19	109-126
ATP	Total creatine	mol/mol	0.16 ± 0.01	0.16 ± 0.02	0.20-0.23
Phospho-creatine			0.54 ± 0.03	0.52 ± 0.05	0.66-0.71

NOTE. Data are the mean ± SD. The 95% confidence intervals for the healthy reference group are unpublished data, 1993 (Gamrin L, Berg H, and Hultman E).

content during the comparatively short period covered by the present study.

Concentrations of energy-rich phosphates are low in muscle of ICU patients,[4,7-9] and ATP has been reported to decrease further up to 30 days after trauma or burn injury.[21] The most characteristic feature of energy metabolism in critically ill patients is the low phosphorylation fraction of creatine, which did not change over time. Furthermore, the concentration of ATP correlated inversely with the concentration of glutamate ($R^2 = .82$, $P < .05$), which may indicate ineffectiveness of the tricarboxylic acid cycle in the mitochondria. Accumulation of glutamate in parallel with a shortage of energy-rich phosphagens shows that energy production is not functioning according to normal regulation.

The pronounced depletion of free glutamine is perhaps the most characteristic biochemical alteration of skeletal muscle in critically ill patients. Here, the initial biopsy specimen showed a concentration of glutamine that was reduced to 25% of the levels in healthy individuals.[17] Furthermore, this level remained unaltered throughout the course of the disease regardless of disease progression, which also has been recently reported in a group of mixed ICU patients.[22] Obviously, glutamine depletion is already fully established on day 3 in the ICU even in previously healthy individuals. This is a much more severe depletion than is seen following major surgical trauma, which results in a 30% to 50% decrease on postoperative day 3.[17] The establishment of a low glutamine level without any further decline points to a regulatory mechanism that operates at full strength from the start. Stress hormones are known to influence the concentration of glutamine.[23] This influence develops relatively slowly and also continues when hormone levels return to normal.[24] On the other hand, the return of muscle glutamine to normal levels is also a slow process.[24]

Concentrations of BCAA and phenylalanine increased between the two biopsies. The accumulation of these amino acids over time seems to be a characteristic feature of the critically ill patient (Fig 2), and it contrasts with the low but unaltered glutamine concentration. The changes in BCAA and phenylalanine concentrations probably reflect the continuous proteolysis to provide amino acid substrates for export to other tissues. The increase of BCAA and phenylalanine and the reduction of glutamine concentrations have been reported by others,[1-3] but the continuous increase is a new finding that may be used as a marker of the efficacy of nutritional intervention.

The fat concentration increased over time in muscle in relation to FFS. The critically ill patient apparently stores at least part of the supplied nutrients as fat. Even though nutrition was dimensioned according to the measured energy expenditure of the individual patient, there is still the possibility of "overfeeding," or at least of the body not effectively using the supplied nutrients. This is in agreement with findings from other investigators who have measured whole-body composition and found increased levels of fat over time in conventionally nourished critically ill patients.[25]

The total muscle water content in relation to FFS was not affected by the duration of critical illness. It is increased as compared with levels in healthy controls, due to a doubling of the extracellular water.[4,26] These changes were present at the first biopsy and remained at the same level at the second biopsy. There was a correlation between the level of extracellular water and the concentration of aspartate ($P < .05$, $R^2 = .48$) and glutamate ($P < .05$, $R^2 = .54$), not surprisingly the free amino acids with the highest gradients between the intracellular and extracellular space. However, during the ICU stay, the change in the concentration of aspartate correlated with the change in extracellular water ($P < .05$, $R^2 = .48$), which may reflect interference with the energy status of the cell. Since the patients were administered diuretics to maintain fluid balance, the consistency of the total water content, although on an elevated level, was expected. However, it is noteworthy that the intracellular water as related to FFS was reduced below the 95% confidence interval of the reference group at the second biopsy, although there was no significant decrease between the two biopsies. On a whole-body basis, the loss of intracellular water is reported to be greater than would be explained by the protein losses.[27] This favors the concept of a progressive cellular dehydration, which may be involved in the regulation of protein metabolism.[28]

In summary, the protein content of muscle tissue as related to DNA decreased over time in the individual patient. The decrease was of a magnitude of 10% per 5 days of stay in the ICU. Also, the concentration of energy-rich compounds in relation to the tissue dry weight or protein content was related to the length of critical illness. Among the free amino acids, the pronounced depletion of glutamine exhibited a remarkably constant level, whereas the concentrations of BCAA and phenylalanine increased over time. These parameters may therefore be used as biochemical markers related to muscle tissue depletion. In general, it is preferable to use a battery of biochemical parameters in parallel rather than a single marker. A period of 5 days seems sufficient to detect an effect on these biochemical markers in muscle.

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