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Protein Metabolism in Specific Tissues of Endotoxin-Treated Rats: Effect of Nutritional Status

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Summary

Rats received an injection of [^{14}C]leucine and were then divided into four groups. Groups I and II consisted of *ad libitum* fed rats were administered saline or endotoxin of *Salmonella enteritidis* eight and twenty-two h after the [^{14}C]leucine treatment. Animals of Group III (saline) and Group IV (endotoxin) fasted after [^{14}C]leucine injection. Twenty three hours after [^{14}C]leucine treatment rats were injected with [^{3}H]leucine and sacrificed 20 min afterwards. Endotoxin administration decreased body weight in fed rats only. After endotoxin treatment, higher [^{3}H]leucine specific activity in the blood plasma, decreased leucine incorporation into proteins and lowered plasma amino acid levels were observed. [^{14}C]leucine radioactivity was significantly higher in the spleen and lower in skeletal muscles of endotoxin-treated rats. All changes were less expressed in fasted than in *ad libitum* fed animals. Our results indicate that endotoxin treatment results in (a) changes in host metabolism that are not mediated solely by anorexia; (b) a decrease of protein synthesis in the viscera and skeletal muscles; (c) an increase of protein degradation in skeletal muscles; (d) reutilization of leucine released from skeletal muscles in viscera, and (e) a slower disappearance rate of leucine from the blood.

Key words

Endotoxin – Cachexia – Leucine – Protein metabolism – Sepsis

Introduction

The acute metabolic response to trauma or severe infection includes anorexia, weight loss, enhanced nitrogen excretion and muscle wasting. Accelerated release of amino acids from skeletal muscles and transfer to the liver for gluconeogenesis and protein synthesis appear to be responses that are essential for survival (Rosenblatt *et al.* 1983, Andus *et al.* 1991). However, this beneficial response of the body is disastrous if it is excessive or if it lasts for a long time. It is now recognized that cytokines are the principal mediators of this important metabolic reaction (Cerami *et al.* 1985).

Despite many elegant and detailed studies, there are numerous controversial issues. Primarily, there is disagreement about the significance of anorexia in the development of cachexia. Some studies provide evidence that metabolic abnormalities involved in the pathogenesis of cachexia in cancer, sepsis and after endotoxin or cachectin administration (Lundholm *et al.* 1981, Costa *et al.* 1981, Karlstad and Sayeed 1987, Tracey *et al.* 1990) are not associated with inadequate food intake. In other studies, it was suggested that nitrogen loss was not increased and that weight loss could be accounted for by anorexia (Mahoney and Tisdale 1988, Michie *et al.* 1989). In addition, reports of the effects on protein synthesis in skeletal muscle are contradictory, with increased (Ryan 1976), unchanged (Clark *et al.* 1984, Hasselgren *et al.* 1987), or decreased synthetic rates being reported (O'Keefe *et al.* 1974, Rennie and Harrison 1984).

Administration of bacterial endotoxin elicits systemic manifestations observed in subjects with septicaemia. These changes are not direct effects of the endotoxin, but are mediated by potent cytokines

released by macrophages and probably other cells (Cerami *et al.* 1985, Nathan 1987). The purpose of the present study was to assess alterations in protein synthesis and protein breakdown in specific tissues in fed and fasted animals during endotoxin treatment.

Methods

Male Wistar rats weighing between 160–185 g were obtained from VELAZ, Prague. Rats were housed in standardized cages in rooms with controlled temperature, and 12 h light-dark cycle and received Velaz-Altromin 1320 laboratory chow (VELAZ, Prague) and drinking water *ad libitum*. All procedures involving animals were performed according to the guidelines set by the Institutional Animal Use and Care Committee of the Charles University. [1-^{14}C]leucine (1.85 GBq/mmol) and [4,5-^{3}H]leucine (1.7 TBq/mmol) were obtained from Amersham International (Buckinghamshire, UK). Folin-Ciocalteu phenol reagent, *Salmonella enteritidis* endotoxin and bovine serum albumin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Remaining chemicals were obtained from Lachema (Brno, CZ). The radioactivity of the samples was measured with the liquid scintillation radioactivity counter LS 6000 (Beckman Instruments, Fullerton, CA, USA).

Protocol of study. Between 0900 and 0930 h rats received an intravenous injection of 1-[^{14}C]leucine in a dose 22.5 μCi/kg and then were randomly divided into four groups. Groups I and II consisting of *ad libitum* fed rats received saline or lipopolysaccharide of *Salmonella enteritidis* (endotoxin) in a dose of 5 mg/kg (Nawabi *et al.* 1990) eight and twenty-two hours after [1-^{14}C]leucine treatment. Animals of Group III (saline) and Group IV (endotoxin) fasted after [1-^{14}C]leucine injection and were treated using an identical scheme as used for *ad libitum* fed animals. All animals had free access to drinking water. Food intake was measured in both groups of fed animals. Twenty-three hours after [1-^{14}C]leucine treatment, rats were injected intravenously with 375 μCi/kg of [4,5-^{3}H]leucine and sacrificed by blood withdrawal from the aorta 20 min afterwards. Samples of liver, gastrocnemius muscle, small intestine, spleen, left kidney and heart were quickly removed and immediately frozen in liquid nitrogen. Samples of blood and blood plasma were processed immediately, samples frozen in liquid nitrogen were processed within 2 weeks.

Haematological examination. Blood was mixed in a tube containing K_3EDTA (1.5 mg/ml of blood). The blood count was evaluated using a blood particles analyzer Coulter Counter JT3 (Coulter Electronics, Luton, UK).

Amino acid concentrations and leucine specific activity in blood plasma. Amino acid concentrations in the plasma and tissue homogenates were determined after deproteinization of the samples by sulphosalicylic acid on an automatic analyzer of amino acids T339 (Mikrotechna, Prague). One hundred microlitres of supernatant obtained after deproteinization of blood plasma samples was used for measurement of radioactivity. Radioactivity was corrected on the basis of plasma free leucine concentration and expressed as leucine specific activity.

Protein synthesis and degradation in muscle and viscera. Small pieces of tissue (about 0.5 g) were rinsed and homogenized in 2 % $HClO_4$. The precipitated proteins were collected by centrifugation. The pellet was washed three times in $HClO_4$ and hydrolyzed in 2 N NaOH at 60 °C for 3 h. One hundred microlitres solution was used for measurement of radioactivity. Protein synthesis was estimated from incorporation of [4,5-^{3}H]leucine into proteins and calculated on the basis of leucine specific activity. Results are expressed as nmol leucine/20 min. Protein degradation was evaluated by measuring the decline of radioactivity in tissue proteins prelabelled by [1-^{14}C]leucine. Protein concentration was determined by the method of Lowry *et al.* (1951).

Statistical analysis. Results are presented as the mean ± S.E.M. Significance of differences of the means was checked using analysis of variance and then by Bonferroni's t-test. $P<0.05$ was considered significant.

Table 1
Changes in erythrocyte, leucocyte, and platelet number after endotoxin treatment of fed and fasted rats.

	Fed animals		Fasted animals	
	Saline	Endotoxin	Saline	Endotoxin
Erythrocytes (x 10^{12}/l)	6.5±0.2	5.1±0.7	6.6±0.3	5.2±0.6
Leucocytes (x 10^{9}/l)	3.3±0.3	1.8±0.8	4.7±0.8	2.4±0.3
Platelets (x 10^{9}/l)	830±30	138±75*	869±46	285±144*

*Mean ± S.E.M., (n=6 in each group). *P<0.05, endotoxin vs. saline treated rats*

Results

Endotoxin treatment resulted in a slight decrease of leucocytes and a profound decrease of platelet number in the blood (Table 1). The decrease of leucocyte observed may reflect leucocyte adhesion to endothelial surfaces (Tracey and Cerami 1990), and/or efflux of leucocytes from peripheral blood and their sequestration in the tissues, primarily in the lungs and liver (Toft *et al.* 1994). Reduction in circulating platelet number associated with endotoxin treatment results from disseminated intravascular coagulation (Emerson *et al.* 1987). Changes in red blood cell number were not significant.

In *ad libitum* fed animals, a significant decrease of body weight and reduced food intake to 25-30 % were observed after the endotoxin treatment. However, no effect of endotoxin on changes of body weight was observed in fasted animals. Endotoxin administration resulted in a significant increase of spleen weight and in a relative increase (per kg of b.w.) of kidney weight. We did not observe significant differences in the weight of liver and heart at the end of experiment (Table 2).

Table 2
Changes of body weight and weights of heart, spleen, kidney and liver after endotoxin treatment of fed and fasted rats.

Tissue		Fed animals		Fasted animals	
		Saline	Endotoxin	Saline	Endotoxin
Body weight	- initial (g)	177.5±3.4	177.5±5.0	180.0±4.7	181.0±2.5
	- final (g)	184.0±3.0	174.0±4.2	170.0±4.0	171.0±2.8
	- % of initial	103.8±1.2	98.2±0.9*	94.5±0.7#	94.5±1.5
Liver	- g	6.95±0.30	6.56±0.32	5.29±0.27#	5.96±0.39
	- g/kg b.w.	37.7±1.3	37.7±1.9	31.2±1.5	34.9±2.2
Spleen	- g	0.51±0.03	0.60±0.01*	0.46±0.02	0.58±0.03*
	- g/kg b.w.	2.8±0.1	3.4±0.1*	2.7±0.1	3.4±0.2*
Kidney	- g	0.61±0.02	0.67±0.02	0.60±0.01	0.65±0.01
	- g/kg b.w.	3.3±0.1	3.9±0.1*	3.5±0.1	3.8±0.1
Heart	- g	0.61±0.03	0.62±0.02	0.59±0.02	0.60±0.03
	- g/kg b.w.	3.3±0.1	3.6±0.1	3.5±0.1	3.5±0.1

*Mean ± S.E.M.; (n=6 in each group). *P<0.05, endotoxin vs. saline treated rats; #P<0.05, fasted vs. fed animals*

Table 3 presents significantly higher [^{3}H]leucine specific activity in the blood plasma of endotoxin-treated rats. Leucine incorporation into proteins after endotoxin treatment decreased significantly in all observed tissues of *ad libitum* fed animals. The highest decrease was observed in skeletal muscles. The decrease of leucine incorporation was less expressed in fasted animals.

[1-^{14}C]leucine radioactivity (Table 4) was significantly higher in the spleen of endotoxin-treated rats. However, no difference was observed when the results were expressed per mg protein or per gram spleen tissue. On the contrary, lower ^{14}C-leucine radioactivity was found in skeletal muscles of endotoxin-treated rats.

Table 5 shows a significant decrease in plasma levels of most amino acids in endotoxin-treated rats. A profound decrease was observed primarily in glutamine, proline, glycine, alanine, citrulline, methionine, valine, leucine and isoleucine. Changes in fasted animals were less pronounced than those in *ad libitum* fed rats.

Table 3
[^{3}H]leucine specific activity in blood plasma and the incorporation into proteins after endotoxin treatment in fed and fasted rats

Tissue	Fed animals		Fasted animals	
	Saline	Endotoxin	Saline	Endotoxin
Plasma				
dpm.10^3/μmol	4158±258	8878±979*	4725±343	750± 507*
Skeletal muscle				
nmol/mg protein	0.09±0.01	0.03±0.00*	0.06±0.00	0.03±0.00*
nmol/g	7.71±0.77	2.61±0.28*	6.19±0.47	2.84±0.24*
Liver				
nmol/mg protein	0.38±0.04	0.25±0.03*	0.32±0.03	0.25±0.02
nmol/g	49.5±4.59	28.2±2.65*	42.9±2.63	32.2±2.90
nmol	345±36.4	186±21.4*	226±17.0#	191±18.9
nmol/kg b.w.	1870±200	1070±120*	1320±85	1130±129
Spleen				
nmol/mg protein	0.19±0.02	0.10±0.01*	0.12±0.01#	0.11±0.01
nmol/g	29.7±2.86	16.4±1.61*	25.0±1.46	21.4±1.63
nmol	15.1±1.25	9.7±0.79*	11.5±0.97	12.2±0.52
nmol/kg b.w.	82.1±7.4	55.4±4.5*	68.0±6.3	71.9±3.9
Kidney				
nmol/mg protein	0.36±0.03	0.22±0.02*	0.30±0.03	0.24±0.02
nmol/g	50.3±3.24	26.5±2.50*	46.0±3.40	32.3±2.85*
nmol	31.1±2.85	17.9±1.83*	27.5±1.96	20.9±1.65
nmol/kg b.w.	169±15.0	102±8.9*	163±13.7	123±11.1
Heart				
nmol/mg protein	0.23±0.02	0.10±0.01*	0.16±0.01#	0.10±0.01*
nmol/g	19.8±1.03	10.4±0.98*	17.4±0.80	12.3±1.15*
nmol	12.1±0.96	6.5±0.67*	10.2±0.25	7.3±0.56*
nmol/kg b.w.	65.8±5.4	36.9±3.5*	60.5±2.6	43.2±3.7*
Small intestine				
nmol/mg protein	0.48±0.05	0.28±0.03	0.41±0.03	0.36±0.14
nmol/g	52.2±4.52	28.5±2.83*	43.0±3.00	35.3±3.35*

*Mean ± S.E.M., (n=6 in each group). *P<0.05, endotoxin vs. saline treated rats; #p<0.05, fasted vs. fed rats.*

Discussion

All methods currently used for measuring protein breakdown and synthesis involve various assumptions and limitations which make absolute rates of protein turnover potentially inaccurate (Rennie 1985). In the present study, we were interested in the differences of the proteolysis and protein synthesis in individual tissues rather than in absolute rates of protein degradation. Our simple procedure enables us to estimate the contributions of specific tissues to whole body changes and reutilization of leucine released from other tissues of the body. However, the changes of radioactivity in proteins were not followed at various intervals during the period of experiment. Since protein turnover, protein synthesis and proteolysis might not have occurred at an even rate during the whole experimental period, the obtained results should be interpreted with caution.

Effect of nutritional status

A significant decrease of body weight and food intake reduced to 25–30 % were observed in *ad libitum* fed endotoxin-treated rats. Furthermore, no effect of endotoxin treatment on body weight was observed in fasted animals and both decreased food intake and endotoxin treatment resulted in a decrease of protein synthesis. It thus appears that the major part of weight loss in endotoxaemia is due to anorexia. However, endotoxin treatment resulted in a significant decrease of protein synthesis in skeletal muscles and hearts of fasted rats. In addition, ^{14}C-leucine radioactivity was significantly lower in skeletal muscle and higher in the kidney and spleen. These results demonstrate that the effects of endotoxin on protein metabolism are not only due to anorexia and that the principle alteration in protein metabolism elicited by endotoxin treatment is accelerated protein breakdown in skeletal muscle.

Table 4
[^{14}C]leucine radioactivity in proteins of gastrocnemius muscle, liver, heart, spleen, kidney and small intestine after endotoxin treatment of fed and fasted rats

Tissue	Fed animals		Fasted animals	
	Saline	Endotoxin	Saline	Endotoxin
Skeletal muscle				
dpm/mg protein	75.2±4.39	55.1±3.83*	68.5±4.49	50.4±1.64*
dpm.10^3/g	6.43±0.32	5.40± 0.20*	6.18±0.39	5.72±0.97
Liver				
dpm/mg protein	128±9.0	152±9.3	152±11.3	165±6.6
dpm.10^3/g	16.8±1.06	17.4±0.22	20.5±0.96#	21.3±0.49#
dpm.10^3	116±8.11	114±6.27	109±8.62	127±8.75
dpm.10^3/kg b.w.	633±44.5	658±34.5	641±46.7	747±52.3
Spleen				
dpm/mg protein	94.6±1.11	87.0±2.5	83.4±3.90	86.2±2.87
dpm.10^3/g	15.2±0.75	14.9±0.65	17.8±0.67	17.2±0.61
dpm.10^3	7.73±0.11	8.82±0.26*	8.10±0.29	10.00±0.59*
dpm.10^3/kg b.w.	42.0±0.96	50.7±1.76*	47.7±1.98	58.6±2.84*
Kidney				
dpm/mg protein	132±4.38	139±7.52	120±9.37	159±12.00
dpm.10^3/g	18.6±0.74	16.7±0.78	19.2±1.00	21.7±1.13#
dpm.10^3	11.4±0.26	11.2±0.58	11.5±0.62	14.2±0.85#
dpm.10^3/kg b.w.	61.8±1.64	64.4±2.46	67.6±3.86	83.0±4.82#
Heart				
dpm/mg protein	143±10.90	107±10.60	113±7.47	113±8.45
dpm.10^3/g	12.1±0.79	11.3±0.31	12.7±0.51	13.1±0.84
dpm.10^3	7.29±0.38	7.01±0.31	7.51±0.37	7.83±0.29
dpm.10^3/kg b.w.	39.6±2.08	40.2±1.24	44.3±2.30	46.0±2.29
Small intestine				
dpm/mg protein	270±25.0	270±15.0	319±17.6	320±22.0
dpm.10^3/g	29.8±3.58	27.3±1.69	33.5±1.50	31.7±2.14

*Mean ± S.E.M., (n=6 in each group). *P<0.05, endotoxin vs. saline treated rats; #p<0.05, fasted vs. fed rats.*

Table 5
Amino acid concentrations in blood plasma after endotoxin treatment in fed and fasted rats.

Amino acid (μmol/l)	Fed animals		Fasted animals	
	Saline	Endotoxin	Saline	Endotoxin
Taurine	102±18.8	153±41.0	125±20.6	157±33.0
Aspartate	8.8±3.0	9.6±1.7	7.2±3.2	11±0.9
Threonine	146±13.4	79±9.1*	137±20.3	95±8.3
Serine	123±9.0	61±6.1*	117±19.2	82±7.6
Asparagine	62±7.1	18±3.5*	43±3.4	24±2.3*
Glutamate	45±2.6	27±3.1*	43±4.3	36±4.3
Glutamine	564±23.0	180±17.1*	350±30.4#	180±12.4*
Proline	240±21.9	95±10.1*	175±11.1#	95±10.1*
Glycine	297±28.8	103±15.3*	285±32.6	121±9.3*
Alanine	445±45.8	235±21.9*	331±40.5	210±16.9*
Citrulline	95±5.6	40±5.8*	67±9.7#	34±4.5*
Valine	180±14.6	98±11.0*	171±19.1	123±12.2
Leucine	129±10.9	78±7.8*	140±11.8	103±5.9*
Cystine	41±4.3	35±3.0	42±6.1	33±2.3*
Methionine	57±2.7	27±2.3*	44±2.9#	22±0.8*
Isoleucine	83±7.5	43±3.7*	92±5.6	54±3.8*
Tyrosine	57±1.7	39±5.0*	65±7.5	44±3.7
Phenylalanine	54±2.7	48±3.4	59±5.1	51±3.3
Tryptophan	57±3.3	42±5.7	61±8.5	39±2.3
Ornithine	60±2.6	54±10.1	40±4.5#	39±3.7
Lysine	218±18.5	132±6.6*	166±15.5	136±7.9
Histidine	47±5.1	38±2.5	48±8.1	35±1.7
Arginine	33±6.0	68±5.0*	68±22.5	61±5.3
Total amino acids	3357±169	1748±136*	2860±235	1834±112*

*Mean ± S.E.M., (n=6 in each group). *P<0.05, endotoxin vs. saline treated rats; #P<0.05, fasted vs. fed animals*

Effect on protein metabolism in specific tissues

Our experimental data reveal that the changes elicited by endotoxin treatment are quite different in skeletal muscles and the viscera. Significantly lower [1-^{14}C] leucine radioactivity after endotoxin treatment when compared to saline-treated controls, indicating increased protein breakdown, was observed in skeletal muscle. In addition, the highest decrease of protein synthesis after endotoxin treatment was observed in skeletal muscles when compared to other tissues. Our study is in good agreement with other findings demonstrating muscle wasting during sepsis, injury, cancer cachexia or after administration of bacterial endotoxins in animals. Accelerated proteolysis and inhibited protein synthesis contribute to the increased flux of amino acids from skeletal muscle. This metabolic alteration serves the purpose of providing the viscera with increased amounts of amino acids, mainly for protein synthesis and gluconeogenesis.

Significantly higher values of ^{3}H-leucine specific activity in the blood plasma of endotoxin-treated rats indicate that the disappearance rate of leucine from the blood is slower. This result is consistent with observations of a marked decrease of protein synthesis both in muscle and the viscera estimated by ^{3}H-leucine incorporation after endotoxin treatment. However, these data are not consistent with experimental data suggesting that protein synthesis in the viscera is accelerated during sepsis or injury (Ryan 1976, Rosenblatt *et al.* 1983). The possible explanation of this disagreement may be that the response of the organism to the second injection of endotoxin (i.e. period in which we measured the rate of protein synthesis) is less pronounced (Tracey *et al.* 1988). We suppose that an increase of protein synthesis in the viscera occurred after the first injection of endotoxin.

This explanation may be supported by increased ^{14}C-leucine radioactivity in the spleen. The increase of ^{14}C-leucine radioactivity was associated with an increase of spleen weight and protein content (data not shown) indicating reutilization of leucine released from other tissues.

Virtually all amino acid levels decreased as a result of endotoxin treatment in our study both in fed and fasted rats. This observation is in good agreement with the decrease of plasma amino acid levels in septic patients (Clowes *et al.* 1980). Considering the marked decrease of protein synthesis and slower disappearance rate of leucine from the blood, it is possible that this phenomenon is due to an increased oxidation of amino acids as fuel and/or conversion to other substrates through processes such as gluconeogenesis or ketogenesis. Increased leucine oxidation has repeatedly been reported in sepsis, trauma or after cytokine administration (Mizock 1985, Nawabi *et al.* 1990, Holeček *et al.* 1992). It can be suggested that the increase of leucine oxidized fraction associated with a decrease of leucine turnover (deduced from the reduced leucine disappearance from the blood plasma and decreased leucine incorporation into proteins) is the predominant mechanism lowering plasma leucine levels in our model of endotoxaemia. This mechanism may explain the decrease in plasma concentrations of the remaining branched-chain amino acids (i.e. valine and isoleucine) and probably the decrease of most of the other plasma amino acids.

We assume that the decrease of glutamine level is primary clinical importance. It has been shown that glutamine is utilized at a very high rate by the intestine (Windmueller and Spaeth 1974), kidneys (Squires *et al.* 1976) and cells of the immune system (Newsholme *et al.* 1988). Consequently, the decrease in plasma glutamine may contribute to the state of immunosuppression (Parry-Billings *et al.* 1990). An adequate supply of glutamine prevents the malfunction of the small intestine and enhances survival following injury (Wolfe *et al.* 1989). Thus in severe trauma, sepsis and other conditions, the preservation of normal glutamine concentrations may be necessary.

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References

ANDUS T., BAUER J., GEROK W.: Effects of cytokines on the liver. *Hepatology* **13:** 369–375, 1991.

CERAMI A., IKEDA Y., TRANG N.L.E., HOTEZ P.J., BEUTLER B.: Weight loss associated with an endotoxin-induced mediator from peripheral macrophages: the role of cachectin (tumor necrosis factor). *Immunol. Lett.* **11:** 173–177, 1985.

CLARK A.S., KELLY R.A., MITCH W.E.: Systemic response to thermal injury in rats: accelerated protein degradation and altered glucose utilization in muscle. *J. Clin. Invest* **74:** 888–897, 1984.

CLOWES G., RANDALL H., CHA C.: Amino acid and energy metabolism in septic and traumatized patients. *J. Parent. Ent. N*utr. **4:** 195–205, 1980.

COSTA G., BEWLEY P., ARAGON M., SIEBOLD J.: Anorexia and weight loss in cancer patients. *Cancer Treat. Rep.* **65** (Suppl 5): 3–7, 1981.

EMERSON T.E., FOURNEL M.A., LEACH W.J., REDEUS T.B.: Protection against disseminated intravascular coagulation and death by anti-thrombin III in the *Escherichia coli* endotoxemic rat. *Circ. Shock* **21:** 1–13, 1987.

HASSELGREN P.-O., WARNER B.W., JAMES J.H., TAKEHERA H., FISCHER J.E.: Effect of insulin on amino acid uptake and protein turnover in skeletal muscle from septic rats. Evidence for insulin resistance of protein breakdown. *Arch. Surg.* **122:** 228–233, 1987.

HOLEČEK M., PAUL H.S., ADIBI S.A.: Activation of muscle branched-chain keto acid dehydrogenase (BCKAD) in diabetic and tumor necrosis factor treated rats. *Clin. Nutr.* **11** (Suppl.): 84–85, 1992.

KARLSTAD M.D., SAYEED M.M.: Effect of endotoxic shock on skeletal muscle intracellular electrolytes and amino acid transport. *Am. J. Physiol.* **252:** R674–R680, 1987.

LOWRY O.H., ROSENBROUGH N.J., FARR A.L., RANDALL R.J.: Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275, 1951.

LUNDHOLM K., EDSTRÖM S., EKMAN L., KARLBERG I., SCHERSTÉN T.: Metabolism in peripheral tissues in cancer patients. *Cancer Treat. Rep.* **65** (Suppl 5): 79–83, 1981.

MAHONEY S.M., TISDALE M.J.: Induction of weight loss and metabolic alterations by human recombinant tumor necrosis factor. *Br. J. Cancer* **58:** 345–349, 1988.

MICHIE H.R., SHERMAN M.L., SPRIGGS D.R., ROUNDS J., CHRISTIE M., WILMORE D.W.: Chronic TNF infusion causes anorexia but not accelerated nitrogen loss. *Ann. Surg.* **209:** 19–24, 1989.

MIZOCK B.A.: Branched-chain amino acids in sepsis and hepatic failure. *Arch. Intern. Med.* **145:** 1284–1288, 1985.

NATHAN C.F.: Secretory products of macrophages. *J. Clin. Invest.* **79:** 319–326, 1987.

NAWABI M.D., BLOCK K.P., CHAKRABARTI M.C., BUSE M.G.: Administration of endotoxin, tumor necrosis factor, or interleukin 1 to rats activates skeletal muscle branched-chain α-keto acid dehydrogenase. *J. Clin. Invest.* **85:** 256–263, 1990.

NEWSHOLME E.A., NEWSHOLME P., CURI R., CHALLONER E., ARDANI M.S.M.: A role for muscle in the immune system and its importance in surgery, trauma, sepsis and burns. *Nutrition* **4:** 261–268, 1988.

O'KEEFE S.J.D., SENDER P.M., JAMES W.P.T.: 'Catabolic' loss of body nitrogen in response to surgery. *Lancet* **2:** 1035–1038, 1974.

PARRY-BILLINGS M., EVANS J., CALDER P.C., NEWSHOLME E.A.: Immunosuppression following major burns: could glutamine play a role? *Lancet* **336:** 523–525, 1990.

RENNIE M.J.: Muscle protein turnover and the wasting due to injury and disease. *Br. Med. Bull.* **41:** 257–264, 1985.

RENNIE M.J., HARRISON R.: Effects of injury, disease, and malnutrition on protein metabolism in man: unanswered questions. *Lancet* **1:** 323–325, 1984.

ROSENBLATT S., CLOWES G.H.A., GEORGE B.C., HIRSCH E., LINDBERG B.: Exchange of amino acid by muscle and liver in sepsis. *Arch. Surg.* **118:** 167–175, 1983.

RYAN N.T.: Metabolic adaptations for energy production during trauma and sepsis. *Surg. Clin. N. Am.* 56: 1073–1090, 1976.

SQUIRES E.J., HALL D.E., BROSNAN J.T.: Arteriovenous differences for amino acids and lactate across kidney of normal and acidotic rats. *Biochem. J.* **160:** 125–128, 1976.

TOFT P., LILLEVANG S.T., TONNESES E., NIELSEN C.H., RASMUSSEN J.W.: The redistribution of granulocytes following E. coli endotoxin induced sepsis. *Acta Anaesth. Scand.* **38:** 852–857, 1994.

TRACEY K. J., CERAMI A.: Metabolic response to cachectin-TNF. A brief review. *Ann. N.Y. Acad. Sci.* 587: 325–331, 1990.

TRACEY K.J., LOWRY S., CERAMI A.: Cachectin: A hormone that triggers acute shock and chronic cachexia. *J. Infect. Dis.* **157:** 413–420, 1988.

TRACEY K.J., MORGELLO S., KOPLIN B., FAHEY T.J., FOX J., ALEDO A., MANOGUE K.R., CERAMI A.: Metabolic effects of cachectin/tumor necrosis factor are modified by the site of production: cachectin/tumor necrosis factor-secreting tumor in skeletal muscle induces chronic cachexia, while implantation in brain induces predominantly acute anorexia. *J. Clin. Invest.* **86:** 2014–2024, 1990.

WINDMUELLER H.G., SPAETH A.E.: Uptake and metabolism of plasma glutamine by the small intestine. *J. Biol. Chem.* **249:** 5070–5079, 1974.

WOLFE R.R., JAHOOR F., HARTL W.H.: Protein and amino acid metabolism after injury. *Diabetes Metab. Rev.* **5:** 149–164, 1989.

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