

# Effects of Brief Starvation on Muscle Amino Acid Metabolism in Nonobese Man

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**ABSTRACT** A reduction in the release of substrate amino acids from skeletal muscle largely explains the decrease in gluconeogenesis characterizing prolonged starvation. Brief starvation is associated with an increase in gluconeogenesis, suggesting increased release of amino acids from muscle. In the present studies, accelerated amino acid release from skeletal muscle induced by brief starvation was sought to account for the accompanying augmentation of gluconeogenesis. To do this amino acid balance across forearm muscles was quantified in 15 postabsorptive (overnight fasted) subjects and in 7 subjects fasted for 60 h.

Fasting significantly reduced basal insulin (11.3–7.5  $\mu$ U/ml) and increased glucagon (116–134 pg/ml). Muscle release of the principal glycogenic amino acids increased. Alanine release increased 59.4%. The increase in release for all amino acids averaged 69.4% and was statistically significant for threonine, serine, glycine, alanine,  $\alpha$ -aminobutyrate, methionine, tyrosine, and lysine. Thus, with brief starvation, muscle release of glycogenic amino acids increases strikingly. This contrasts with the reduction in amino acid release characterizing prolonged starvation. The adaptation of peripheral tissue metabolism to brief starvation is best explained by the decrease in insulin.

## INTRODUCTION

Metabolic studies of obese patients undergoing prolonged therapeutic starvation reveal a reduction in

A preliminary account of this work appeared as an abstract in: 1974. *J. Clin. Invest.* 54: 61a.

Dr. R. T. Moxley was recipient of a National Institutes of Health Special Fellowship FN 02 1F11NS2617 and Dr. T. Pozefsky was a recipient of an American Diabetes Association Research and Development Award.

Received for publication 3 June 1974 and in revised form 26 September 1975.

new glucose synthesis from nitrogenous precursors (1). The attenuation of gluconeogenesis evident after a 4- to 6-wk fast is due to an adaptive decrease in the release of glycogenic amino acids from skeletal muscle (2). Glycogenic amino acids provided exogenously are readily converted to glucose (3, 4). The feasibility of a reduction in gluconeogenesis follows largely from the replacement of glucose by ketone bodies as the major oxidative substrate for the brain (5). In contrast to the reduction in gluconeogenesis characterizing prolonged starvation, an initial increase early in starvation is likely. Glycogenolysis is estimated to account for 70–80% of splanchnic glucose production in the postabsorptive (overnight fasted) state, and gluconeogenesis the remainder (6). Yet, despite depletion of hepatic glycogen within the first 24 h of starvation (7), glucose turnover measured isotopically in both normal weight and obese subjects fasted for 1 wk is reduced only about one-third (8, 9). Studies of splanchnic tissue metabolism by the hepatic venous catheter technique in obese subjects also indicate glucose production to be diminished by no more than one-third after a brief (36–48 h) fast (10). Recently, Garber et al. (11) reported an increase in hepatic gluconeogenesis in nonobese subjects fasted for 3 days. Increased gluconeogenesis early in starvation would require augmentation of substrate delivery from peripheral tissues to the liver. In the present investigation we sought evidence for such a change in peripheral tissue metabolism by studying amino acid balance across muscle of the forearm in the postabsorptive state and after a brief (60 h) fast.

## METHODS

*Subjects.* Studies were performed in 21 normal, nonobese male volunteers. In 15 subjects, forearm tissue metabolism was quantified under basal postabsorptive conditions (after

a 12-h overnight fast). Their mean age was  $28 \pm 2$  (SEM)<sup>1</sup> yr (range 22–42 yr) and their weight averaged  $109 \pm 2\%$  of ideal<sup>2</sup> (range  $96 \pm 120\%$ ). Another group of seven subjects was fasted for 60 h (Table I). (D. B. was studied both in the postabsorptive state and after brief starvation.) Fasting was taken to begin 12 h after the last meal. Subjects were instructed to consume 8 g of NaCl, 8 g of KCl (as a 10% solution), and at least 2,000 ml of water daily. Weights were obtained and the ketone content of freshly voided urine specimens determined semiquantitatively with nitroprusside (Acetest, Ames Co., Inc., Elkhart, Ind.) at 24-h intervals during the fast. There were no significant differences in age or initial weight between overnight and 60-h fasted subjects.

**Forearm technique.** All studies were performed between 8:30 and 10:30 a.m. With the subject supine, a polyethylene catheter was introduced into a large antecubital vein and threaded deep into the forearm tissues toward the wrist. A scalp-vein needle was placed in a superficial vein. Finally, a needle was introduced into the brachial artery opposite the direction of blood flow and a catheter threaded through it into the artery to terminate about 1 cm beyond the needle tip. Blood samples could then be drawn simultaneously from the deep vein draining mainly muscle, from a superficial vein, and from the artery proximal to the infusion site (through the intra-arterial catheter). Evans blue dye was infused into the artery distal to the site of arterial sampling through the space between the outer wall of the arterial catheter and the inner wall of the needle. Brachial arterial plasma (and blood) flow was measured by the continuous infusion indicator-dilution technique (12). Completeness of dye mixing in the arterial circulation was assessed by comparing the dye concentrations in plasma from simultaneously drawn samples of superficial and deep venous blood. Flow values were discarded when these dye concentrations differed by more than 20%. A sphygmomanometer cuff placed about the wrist was inflated above arterial pressure for 5 min before and during each blood collection to exclude blood flow to the hand. Forearm volume was determined between the wrist cuff and humeral epicondyles by water displacement. Room temperature was maintained at 70°F.

Three sets of blood samples were collected from the artery, the deep vein, and the superficial vein in heparinized syringes at intervals of approximately 20 min in all subjects for the measurement of flow. Amino acids were measured in arterial and deep venous plasma from two sets of samples. The balance of amino acids across forearm muscle ( $\dot{Q}_M$ ) was estimated according to the Fick expression:

$$\dot{Q}_M = F(A - DV)$$

where  $F$  is the brachial arterial plasma flow, and  $A - DV$  the metabolite arterio-deep venous concentration difference. Implicit in this calculation is the assumption that all brachial arterial flow is directed toward deep tissues. Flow to superficial tissues is thought to be about 15% of the total (13); therefore  $\dot{Q}_M$  slightly overestimates balance across the deep system.

**Analyses.** Plasma was separated from each blood sample by centrifugation at 4°C. Protein-free supernates of plasma were made with 10% sulfosalicylic acid for the automated determination of amino acids within 1 wk on an Amino

<sup>1</sup> Standard errors are given for all means throughout the paper.

<sup>2</sup> From the Metropolitan Life Insurance tables, 1959.

TABLE I  
Clinical Data on 60-h Fasted Subjects

Subject	Age	Height	Weight		Percent ideal body weight before fasting*
			Initial	Final	
	yr	cm	kg		%
D. C.	24	178	78.1	76.6	110
D. B.	23	165	64.3	61.7	104
E. B.	23	170	62.7	60.8	99
B. Z.	25	178	77.2	72.8	108
D. K.	25	188	87.5	85.0	108
I. H.	26	193	81.4	78.4	94
R. D.	27	183	72.4	71.4	94

\* From the Metropolitan Life Insurance tables, 1959.

Acid Analyzer (model 121, Beckman Instruments, Inc., Palo Alto, Calif.) (14). Glutamine and asparagine elute as a single peak under the chromatographic conditions employed. This combined peak gradually deteriorates in sulfosalicylic acid filtrates of plasma. The rapid analysis of all samples from a single experiment, coupled with the chromatography of paired arterial and venous samples on the same day, made this a trivial source of error. Insulin was measured by a modification of the double antibody technique (15). Glucagon determinations were available in limited numbers; consequently, measurements were made in subjects from the fasted group and then only under basal conditions postabsorptively and after the 60-h fast. Trasylol (FBA Pharmaceuticals, Inc., New York) was added to blood specimens immediately after collection (1,000 KI U/ml blood) and glucagon measured using antibody 30K which is specific for pancreatic glucagon (16). For comparison of forearm tissue metabolism between overnight and 60-h fasted subjects, a two-tailed Student's  $t$  test was used (17).

## RESULTS

**Arterial amino acid concentration.** The concentration of most amino acids in arterial plasma fell with brief starvation (Table II). However, the branched chain amino acids valine, isoleucine, and leucine rose as did  $\alpha$ -aminobutyrate. Similar changes were noted previously in briefly starved obese subjects (10, 18). The glutamine-asparagine peak was unchanged.

**Amino acid balance across muscle.** The balance of several amino acids across muscle became strikingly more negative with starvation (Table III). These were threonine, serine, glycine, alanine,  $\alpha$ -aminobutyrate, methionine, tyrosine, and lysine. Serine, which was taken up by muscle in the postabsorptive state, was released after a 60-h fast. The balance of all amino acids measured totaled  $-434$  nmol/min per 100 ml forearm in the overnight fasted group and  $-725$  nmol/min per 100 ml forearm in the starved group, an increase of 69.4%.

**Brachial arterial flow.** Three measurements of forearm flow were averaged to give a base-line value for

each subject (Fig. 1). Blood flow was significantly higher in 60-h starved than in overnight fasted subjects ( $5.6 \pm 0.95$  vs.  $2.9 \pm 0.25$  ml/min per 100 ml forearm). An increase in forearm flow was observed in obese patients fasted briefly, though the increment was much smaller than in the present study (19). Fasted subjects of normal weight have not been studied previously in this regard.

Changes in the calculated values for  $\dot{Q}_M$  were accounted for mainly by an increase in brachial arterial flow rather than by changes in amino acid  $A - DV$ . The distribution of flow between deep and superficial tissues in 60-h starved subjects is not known. Consequently, the assumption made in calculating  $\dot{Q}_M$  that all flow is directed toward deep tissues, while a reasonable approximation in postabsorptive subjects (13), may lead to an overestimate of  $\dot{Q}_M$  in starved subjects if the increment in flow is directed to tissues other than muscle. Therefore, to investigate further the distribution of brachial arterial flow in starved subjects, forearm

TABLE II  
Effect of a 60-h Fast on Arterial  
Amino Acid Concentration\*

Amino acids	Arterial concentration		P†
	Postabsorptive	60-h fast	
		<i>μmol/liter</i>	
Threonine	128±5	94±7	<0.001
Serine	115±3	96±4	<0.005
Glutamine-asparagine	534±19	493±15	
Proline	180±10	156±15	
Glutamate	86±5	40±4	<0.001
Citrulline	33±1	26±2	<0.005
Glycine	201±5	161±4	<0.001
Alanine	265±19	160±14	<0.001
α-Aminobutyrate	25±2	72±6	<0.001
Valine	231±10	432±18	<0.001
Half-cystine	109±6	91±3	<0.02
Methionine	22±1	17±1	<0.005
Isoleucine	63±2	147±6	<0.001
Leucine	130±5	276±12	<0.001
Tyrosine	54±2	44±2	<0.005
Phenylalanine	51±2	49±1	
Ornithine	57±2	41±3	<0.001
Lysine	183±10	131±7	
Histidine	85±2	78±2	<0.05
Arginine	88±4	54±2	<0.001

\* n = 15 for postabsorptive and 7 for 60-h fasted subjects. Two values from each subject were averaged. Mean±SEM is given.

† Significance of the difference between postabsorptive and 60-h fasted subjects (unpaired *t* test).

TABLE III

Effect of a 60-h Fast on Amino Acid Balance across Deep Forearm Tissues\*

Amino acids	Muscle balance ( $\dot{Q}_M$ )		P‡
	Postabsorptive	60-h fast	
	<i>nmol/min per 100 ml forearm</i>		
Threonine	-29±4	-51±6	<0.01
Serine	+5±3	-23±6	<0.001
Glutamine-asparagine§	-185±25	-215±69	
Proline	-28±7	-24±5	
Glutamate	+71±8	+55±24	
Citrulline	+2±1	0±2	
Glycine	-40±5	-64±6	<0.01
Alanine	-128±8	-204±18	<0.001
α-Aminobutyrate	0±1	-10±3	<0.02
Valine	-11±4	-23±12	
Half-cystine	+27±3	+23±7	
Methionine	-5±1	-10±1	<0.005
Isoleucine	-8±2	-15±3	<0.1
Leucine	-14±4	-26±7	
Tyrosine	-10±2	-15±2	<0.05
Phenylalanine	-10±2	-15±3	
Ornithine	-1±2	-5±3	
Lysine	-36±5	-64±11	<0.05
Histidine	-13±3	-21±6	
Arginine	-21±4	-28±5	

\* n = 15 for postabsorptive and 7 for 60-h fasted subjects. Two base-line determinations were averaged in each subject. Mean±SEM is given.

† Significance of the difference between postabsorptive and 60-h fasted subjects (unpaired *t* test).

§ n = 7 in postabsorptive group.

muscle blood flow was measured by externally monitoring the rate of xenon-133 washout after intramuscular injection in six nonobese males starved for 60 h (20). In these subjects there was a progressive increase in muscle blood flow evident at 36 and 60 h of starvation. The increment at 60 h was 113% the overnight fasted value,<sup>8</sup> agreeing well with the increment of 91% in brachial arterial flow noted in the present studies as measured by the dye dilution technique. These data validate the calculations of  $\dot{Q}_M$  shown in Table III. Since the increase in brachial arterial flow largely supplies muscle, the greater increase noted in our nonobese subjects compared to that previously reported in obesity may be due to a greater forearm muscle mass relative to adipose tissue in subjects of normal weight.

**Hormone concentration.** The base-line concentration of insulin in arterial plasma was  $11.3 \pm 0.78$  μU/ml in

<sup>8</sup> North, W., Z. V. I. Oster, and T. Pozefsky. To be published.

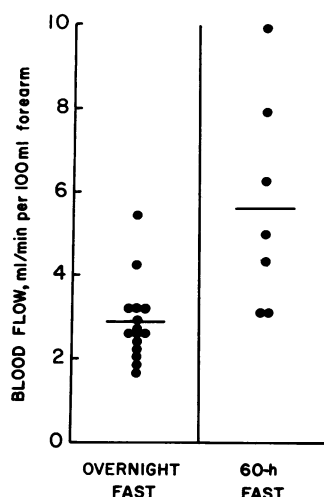


FIGURE 1 Base-line forearm blood flow in 15 overnight and 7 60-h fasted subjects. Means for the two groups are shown by a horizontal line.

the 15 overnight fasted subjects, significantly greater than that of  $7.5 \pm 0.86 \mu\text{U/ml}$  in starved subjects ( $P < 0.005$ , unpaired  $t$  test). In the starved group, a single specimen of venous blood was initially obtained after an overnight fast for the determination of plasma glucagon which was  $116 \pm 20.1 \text{ pg/ml}$ . Glucagon rose slightly to a value in deep venous plasma of  $134 \pm 18.0 \text{ pg/ml}$  after starvation ( $P < 0.05$ , paired  $t$  test).

## DISCUSSION

Skeletal muscle protein is the primary source of glyco-genic amino acids released into the circulation and converted to glucose by the liver (21). Although amino acid release from muscle in overnight fasted man is substantial, its importance to glucose homeostasis is small since no more than 15% of postabsorptive hepatic glucose production can be accounted for by concomitant amino acid uptake (6). Nevertheless, in the absence of dietary intake, continued amino acid release from skeletal muscle at the postabsorptive rate would rapidly lead to nitrogen depletion and loss of muscle mass. Measurements of amino acid balance across forearm muscle after prolonged starvation (2) and in naturally occurring severe protein-calorie malnutrition (22) have revealed an adaptive reduction in muscle amino acid release. In the latter instance, conservation of nitrogen by muscle persists for many weeks after the initiation of refeeding. Undoubtedly, this metabolic adaptation plays an important role in the capacity to survive prolonged periods of nitrogen deprivation.

The present studies call attention to a period early in starvation when nitrogen loss from muscle is actually greater than in the postabsorptive state. The increase

in release of all amino acids averaged 69.4% in subjects starved for 60 h. Release of alanine, the principal nitrogenous glucose precursor, increased 59.4%.<sup>4</sup> It has recently been shown that alanine release from rat diaphragm incubated in vitro (24) as well as from perfused rat hindquarter (25) is increased after 48 h of starvation. Since in the present study three of the amino acids released in increased amounts (threonine, methionine, and lysine) cannot be synthesized *de novo* by muscle, it is likely that accelerated muscle protein breakdown (or decreased synthesis) accounts for the augmentation of amino acid release. The change in lysine balance is particularly noteworthy in this regard. Lysine release has been taken as an index of net protein breakdown because muscle lacks enzymes necessary for its further degradation (26). Studying muscle obtained from the starved rat, Adibi has found a rapid increase in the free intracellular concentration of most essential amino acids. For valine, leucine, and isoleucine, he has attributed the rise in plasma concentration associated with fasting to their increased release from muscle (27). In the present investigation the arterial concentration of branched chain amino acids rose strikingly with starvation as noted previously in briefly fasted man (10, 18). The release of branched chain amino acids from muscle also tended to rise; however,

<sup>4</sup> Although the increases in release of threonine, serine, glycine, alanine,  $\alpha$ -aminobutyrate, methionine, tyrosine, and lysine were statistically significant, muscle balance ( $\dot{Q}_M$ ) was calculated from measurements of amino acids in plasma. Recently, it has been shown that red cells participate in the transport of two of these eight amino acids (alanine and tyrosine) from peripheral to splanchnic tissues (23). To establish that the increase in muscle alanine release attributed to starvation was not an artifact of transport by red cells, the alanine content of whole blood was measured in samples from seven postabsorptive and two of the seven 60-h fasted subjects. The  $\dot{Q}_M$  for alanine based on measurements in whole blood (and thereby accounting for transport in plasma and red cells) was  $-149 \pm 16 \text{ nmol/min per 100 ml forearm}$  for the postabsorptive group, 18% greater than the value of  $-126 \pm 8 \text{ nmol/min per 100 ml forearm}$  based on measurements in plasma obtained from the same blood samples. In seven fasted subjects, the  $\dot{Q}_M$  for alanine based on measurements in plasma of  $-204 \pm 18 \text{ nmol/min per 100 ml forearm}$  (Table III) was significantly greater than that of  $-146 \pm 16 \text{ nmol/min per 100 ml forearm}$  attributable to transport in both the plasma and red cell compartments of postabsorptive subjects ( $P < 0.05$ , unpaired  $t$  test). This, of course, neglects the additional alanine transported by red cells in fasted subjects. In the two fasted subjects in whom whole blood measurements were available,  $\dot{Q}_M$  for alanine averaged  $-299 \text{ nmol/min per 100 ml forearm}$  in contrast to a value in these same subjects from measurements in plasma of  $-233 \text{ nmol/min per 100 ml forearm}$ . Thus, the increase in muscle amino acid release observed in subjects fasted 60 h cannot be attributed to errors introduced by the use of plasma rather than whole blood for amino acid measurements.

changes in their release were not statistically significant. Since muscle is the primary site of branched chain amino acid degradation (28), and their oxidation is enhanced by starvation (29, 30), it is possible that increased release was mitigated by a concomitant increase in their catabolism locally within forearm muscle. Bloxam has shown that in the starved rat the liver may also be a source of branched chain amino acids (31), although the data in man are inconclusive on this point (10).

Felig et al. (10) reported an increase in the splanchnic uptake of glycogenic amino acids after a brief fast in contrast to the reduction occurring after prolonged starvation. It is significant that among the amino acids whose release from muscle increased most consistently with short-term starvation are those most avidly extracted by splanchnic tissues (serine, threonine, glycine, and alanine). Thus, increased delivery of glycogenic amino acids from peripheral tissues to the liver in briefly fasted man contributes to the maintenance of hepatic glucose production at approximately two-thirds the postabsorptive value despite depletion of hepatic glycogen. Muscle glutamine release was large in overnight fasted subjects and unaffected by brief starvation. Glutamine does not, however, appear to be an important substrate for hepatic gluconeogenesis (32).

Short-term starvation was associated with an increase in plasma glucagon as well as a decrease in insulin. These changes undoubtedly influence muscle metabolism. We have recently shown that increments in glucagon of 500–1,500 pg/ml are without effect on skeletal muscle amino acid balance in postabsorptive and 60-h fasted man.<sup>5</sup> Hence, changes in muscle amino acid metabolism characterizing briefly starved man are best explained by the decrease in insulin. A mechanism to account for the ultimate reduction in muscle nitrogen loss accompanying prolonged starvation (2) remains to be elucidated.

#### ACKNOWLEDGMENTS

The authors wish to express their appreciation to Lyle Myerberg and Judy White for their meticulous technical work.

This work was supported in part by U. S. Public Health Service grant AM-14754, and a contract from the Office of Naval Research N00014-67-A-0163-0015.

<sup>5</sup> Pozefsky, T., R. G. Tancredi, R. T. Moxley, J. Dupre, and J. D. Tobin. 1975. Metabolism of forearm tissues in man: Studies with glucagon. *Diabetes*. In press.

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