Origin and Possible Significance of Alanine Production by Skeletal Muscle*

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SUMMARY

These experiments were undertaken to determine the source of alanine released by skeletal muscle and to clarify the possible relationships between this process and the degradation of branched chain amino acids, the release of glutamine, and protein turnover in this tissue.

During incubation in vitro, the rat diaphragm underwent net protein breakdown and released amino acids into the medium at a linear rate. The diaphragm released larger amounts of alanine and glutamine and lower amounts of leucine, isoleucine, and valine than would be expected from the average composition of muscle protein. Addition of the branched chain amino acids increased the production of alanine and glutamine by the diaphragm in a concentration-dependent manner. At the same time, the branched chain amino acids inhibited net protein breakdown; therefore the increased amounts of alanine, glutamate, and lysine must have resulted from de novo synthesis or reduced catabolism of these amino acids. All the other amino acids together failed to increase the production of alanine or glutamate.

Alanine production in muscle appears related to the rapid oxidation of the branched chain amino acids. In diaphragms from fasted rats, both processes occurred at increased rates. Amino groups released on oxidation of branched chain amino acids could account for all nitrogen recovered in alanine. Alanine production by diaphragms from fasted rats increased upon addition of glucose and even further when insulin was present. The muscle incorporated $^{13}$C from [U-$^{13}$C]glucose into alanine, and addition of branched chain amino acids increased the production of [U-$^{13}$C]alanine from [U-$^{13}$C]glucose.

Therefore, alanine production does not reflect protein degradation in muscle but instead appears to be synthesized de novo primarily from exogenous glucose and from amino groups released by catabolism of branched chain amino acids.

These findings suggest the existence of a branched chain amino acid-alanine cycle in the organism. During fasting, enhanced oxidation of branched chain amino acids by muscle would provide energy for muscle, while the concomitant synthesis of alanine from glucose would serve to shuttle ammonia and gluconeogenic precursors back to the liver.

Alanine and glutamine represent over 50% of the amino acids released by skeletal muscle in vivo after an overnight fast (1-4). Previous workers have hypothesized that both alanine and glutamine play key roles as carriers of nitrogen to liver and kidney and as major gluconeogenic precursors. Pozefsky et al. (3) and Marliss et al. (2) have shown that these two amino acids are released by skeletal muscle in greater amounts than could be accounted for by breakdown of muscle protein and they suggested that these amino acids were synthesized de novo by muscle. However in this tissue the origins of the amino groups and the carbon chains of alanine and glutamine have not been identified.

Skeletal muscle metabolizes large amounts of the branched chain amino acids, leucine, isoleucine, and valine (5-7). In addition, the rate of oxidation of these amino acids increased severalfold in muscles from fasted animals (8). The carbon chain of leucine appears to be completely degraded to CO$_2$ by muscle (7), but the fate of the amino group has not been studied. In liver, deamination of amino acids generally results in the formation of urea (4) but this pathway does not occur in skeletal muscle (9). In cell-free extracts from rat muscle, leucine is rapidly transaminated with $\alpha$-keto acids to form glutamate and possibly alanine (10).

The present experiments investigated whether the production of alanine or glutamine by intact muscle might be related to the catabolism of the branched chain amino acids. We have studied the effects of addition of these compounds on the release of alanine, glutamine, and glutamate by isolated diaphragms from fed and fasted rats. Since production of alanine and glutamine may be related to the supply of carbohydrate or to over-all protein balance in the muscle, we have also examined the effects of insulin and glucose on amino acid release by the tissue in vitro.

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¶ Recipient of a Research Career Development Award from the National Institute of Neurological Disease and Stroke, and grants from Muscular Dystrophy Associations of America, the Air Force Office of Scientific Research, and the National Institute of Neurological Diseases.

1 R. Odessey and A. L. Goldberg, manuscript in preparation.
METHODS AND MATERIALS

Male rats weighing 60 to 90 g were obtained from the Charles River Laboratories and maintained as described previously (7). Diaphragms were removed from the animals, dissected free of ribs, and divided into four pieces. Since these muscles release large amounts of amino acids during the first few minutes of incubation in vitro, the pieces of diaphragm were preincubated in Krebs-Ringer bicarbonate buffer containing 10 mM glucose (except where indicated) for 30 min, and were then transferred to fresh medium with or without other additions. Incubations were carried out for 2 or 3 hours as previously described (7). In experiments using [U-14C]glycine, or [1-14C]leucine, [1-14C]isoleucine, and [1-14C]valine (New England Nuclear Corp., Boston, Mass.), the labeled compounds were added only to the incubation medium.

After incubation the muscles were removed, homogenized in trichloroacetic acid, and the supernatant and protein fractions isolated (7). When the diaphragms were incubated with 3H-labeled branched chain amino acids, 14CO2 was collected from the incubation medium and counted as described previously (7). Calculation of the rates of 14CO2 production was based on the specific activity of the amino acids in the medium.

For amino acid analyses, trichloroacetic acid was added to the incubation media and the media centrifuged to remove any protein which may have leaked from the tissue. (This fraction was generally negligible.) The media or acid-soluble fractions from the incubation medium and counted as described previously (7). The media or acid-soluble fractions from different rats were combined and extracted with ether to remove the trichloroacetic acid, and the supernatant and protein fractions were pooled and measured as described previously (7). The media or acid-soluble fractions were performed on the combined pools and media from five quarter diaphragms. No consistent change was observed in the release or production of any other amino acid including glutamine which was normally released amino acids, even though they account for, at most, 20% of the amino acids in protein (Table I). By contrast, the branched chain amino acids were released in low amounts relative to their frequency in protein (Table I) (calculated relative to the amount of tyrosine released from the muscle).

To determine whether the supply of branched chain amino acids could influence the release of other amino acids, rat diaphragms were incubated in medium containing glucose with or without leucine, isoleucine, and valine (Table II). The presence of the branched chain amino acid concentration was increased by 50 to 100% the release of alanine, glutamate, and lysine, and the total amount of these residues in the medium and recovered in the intracellular pool. Similar results were obtained in at least three separate experiments, each of which measured the combined pools and media from five quarter diaphragms. No consistent change was observed in the release or production of any other amino acid including glutamine which was normally released by the diaphragm in greatest amounts.

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Raising the leucine, isoleucine, and valine concentrations in the medium promoted further production of alanine, lysine, and

TABLE I
Comparison of amino acid composition in medium, intracellular pool, and protein after incubation of rat diaphragm

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Released into medium</th>
<th>Recovered in muscle</th>
<th>Present in protein</th>
<th>mol % of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>4.5</td>
<td>1.9</td>
<td>8.1</td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>11.7</td>
<td>10.5</td>
<td>9.5</td>
<td></td>
</tr>
<tr>
<td>Glutamate</td>
<td>7.2</td>
<td>26.2</td>
<td>8.9</td>
<td></td>
</tr>
<tr>
<td>Glutamine</td>
<td>25.2</td>
<td>29.5</td>
<td>12.9*</td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td>4.7</td>
<td>2.1</td>
<td>8.9</td>
<td></td>
</tr>
<tr>
<td>Isoleucine</td>
<td>2.7</td>
<td>1.6</td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td>4.7</td>
<td>1.6</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td>Aspartate</td>
<td>0.8</td>
<td>5.6</td>
<td>9.3*</td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>5.9</td>
<td>0.4</td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td>3.8</td>
<td>0.6</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>Threonine</td>
<td>5.4</td>
<td>2.7</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>Serine</td>
<td>7.0</td>
<td>3.1</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>10.2</td>
<td>11.9</td>
<td>10.3</td>
<td></td>
</tr>
<tr>
<td>Tyrosine</td>
<td>2.9</td>
<td>1.0</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>3.4</td>
<td>1.0</td>
<td>3.6</td>
<td></td>
</tr>
</tbody>
</table>

* Includes glutamate and glutamine.
* Includes asparagine and aspartate.

Reported by others (12). In addition, the intracellular pool of tyrosine and phenylalanine remained constant under these conditions, even though these amino acids were released into the medium at a linear rate (12). Since these two amino acids are neither synthesized nor degraded by skeletal muscle, these observations indicate that net protein degradation occurred in the incubated muscles. Both the synthesis and degradation of proteins are linear under these conditions (12, 13).

Unlike tyrosine and phenylalanine which were recovered in the medium in the same percentage as in muscle protein (Table I), alanine and glutamine together comprised almost 40% of the released amino acids, even though they account for, at most, 20% of the amino acids in protein (Table I). By contrast, the branched chain amino acids were released in low amounts relative to their frequency in protein (Table I) (calculated relative to the amount of tyrosine released from the muscle).

To determine whether the supply of branched chain amino acids could influence the release of other amino acids, rat diaphragms were incubated in medium containing glucose with or without leucine, isoleucine, and valine (Table II). The presence of the branched chain amino acid concentration was increased by 50 to 100% the release of alanine, glutamate, and lysine, and the total amount of these residues in the medium and recovered in the intracellular pool. Similar results were obtained in at least three separate experiments, each of which measured the combined pools and media from five quarter diaphragms. No consistent change was observed in the release or production of any other amino acid including glutamine which was normally released by the diaphragm in greatest amounts.

Raising the leucine, isoleucine, and valine concentrations in the medium promoted further production of alanine, lysine, and

TABLE II
Effects of branched chain amino acids on amino acids from rat diaphragm

Quater diaphragms from fed rats were incubated in medium containing 10 mM glucose. Amino acid analyses of the medium and the acid-soluble fraction were performed on the combined samples from five muscles. Plus signs above column indicate the addition of 0.5 mM leucine, isoleucine, and valine to the medium.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Released into medium</th>
<th>Recovered in muscle</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>0.6</td>
<td>1.0</td>
<td>1.6</td>
</tr>
<tr>
<td>Alanine</td>
<td>1.5</td>
<td>3.2*</td>
<td>4.7</td>
</tr>
<tr>
<td>Glutamate</td>
<td>1.0</td>
<td>1.6*</td>
<td>2.6</td>
</tr>
<tr>
<td>Glutamine</td>
<td>3.3</td>
<td>4.5</td>
<td>8.8</td>
</tr>
<tr>
<td>Aspartate</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Asparagine</td>
<td>0.8</td>
<td>0.8</td>
<td>1.6</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.5</td>
<td>0.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Arginine</td>
<td>0.7</td>
<td>0.6</td>
<td>1.3</td>
</tr>
<tr>
<td>Serine</td>
<td>0.9</td>
<td>0.9</td>
<td>1.8</td>
</tr>
<tr>
<td>Glycine</td>
<td>1.3</td>
<td>1.4</td>
<td>2.7</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.4</td>
<td>0.4</td>
<td>0.8</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.5</td>
<td>0.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Taurine</td>
<td>3.1</td>
<td>3.1</td>
<td>6.2</td>
</tr>
</tbody>
</table>

* The increased amounts of lysine, alanine, and glutamate, and the decreased amounts of tyrosine and phenylalanine were also observed in two additional experiments.
media containing 10 mM glucose with added leucine, isoleucine, and valine (branched chain amino acids), or the other amino acids normally present in plasma. Separate amino acid analyses were performed on the combined media and acid-soluble fractions from diaphragms of five rats. Values represent the sum of the individual amino acids present in the acid-soluble fraction and medium at the end of incubation.

Table III

Effect of exogenous amino acids on production of amino acids by rat diaphragm

Quarter diaphragms from fed rats were incubated for 2 hours in media containing 10 mM glucose with added leucine, isoleucine, and valine (branched chain amino acids), or the other amino acids normally present in plasma. Separate amino acid analyses were performed on the combined media and acid-soluble fractions from diaphragms of five rats. Values represent the sum of the individual amino acids present in the acid-soluble fraction and medium at the end of incubation.

<table>
<thead>
<tr>
<th>Amino acid produced</th>
<th>Amount produced upon incubation with</th>
<th>Branches chain amino acids</th>
<th>Other plasma amino acids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Branched chain amino acids</td>
<td>0.5 mM</td>
<td>1.0 mM</td>
</tr>
<tr>
<td>Totalb.</td>
<td>28.0</td>
<td>28.6</td>
<td>28.6</td>
</tr>
<tr>
<td>Ala + Glu + Lys</td>
<td>5.1</td>
<td>9.2</td>
<td>11.6</td>
</tr>
<tr>
<td>Remaining amino acids</td>
<td>18.6</td>
<td>18.8</td>
<td>17.0</td>
</tr>
<tr>
<td>Alanine</td>
<td>2.1</td>
<td>4.4</td>
<td>5.8</td>
</tr>
<tr>
<td>Glutamate</td>
<td>0.7</td>
<td>1.1</td>
<td>1.3</td>
</tr>
<tr>
<td>Lysine</td>
<td>4.9</td>
<td>5.4</td>
<td>5.1</td>
</tr>
<tr>
<td>Glutamine</td>
<td>0.4</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.5</td>
<td>0.4</td>
<td>0.3</td>
</tr>
</tbody>
</table>

* All amino acids at twice normal plasma concentrations (14) excluding leucine, isoleucine, valine, alanine, glutamate, and glutamine.

b Total includes all amino acids present in media and acid-soluble fractions except for the branched chain amino acids.

glutamate but again had no effect on the production of glutamine (Table III). By contrast, when the remaining amino acids were added to the incubation medium at twice their normal plasma concentrations (14), they failed to stimulate alanine or glutamine production in two experiments (Table III). (The apparent stimulation of glutamate and glutamine production by plasma amino acids shown in Table III was not confirmed in subsequent experiments.) Alanine production was also not stimulated when the incubation medium contained these amino acids at 5 times plasma levels. Thus the branched chain amino acids are the only amino acids found capable of increasing the production of alanine. Additional experiments have shown that other possible nitrogen sources found in serum, including ammonia or adenine, do not increase the production of alanine.

Addition of the branched chain amino acids to the incubation medium reduced in a concentration-dependent manner the total amount of tyrosine and phenylalanine recovered (Table III). Thus leucine, isoleucine, and valine together appear to inhibit net protein breakdown in these muscles, in accord with previous reports (12). Nevertheless, the total amount of free amino acids recovered increased during incubation. This increase reflected the greater amounts of alanine, glutamate, and lysine (Table III) and probably resulted from net synthesis of these amino acids.

Subsequent experiments investigated whether the net production of alanine, glutamate, and lysine is related to the degradation of the branched chain residues. Amino acid release was measured from diaphragms of fasted animals which oxidize leucine, isoleucine, and valine severalfold more rapidly than muscles from fed animals (7). After a 2-day fast, the release of alanine by the diaphragm was 2.3- to 2.8-fold greater than by the muscle from fed controls. This result was obtained both in the presence and absence of the branched chain amino acids (0.5 mM) (Table IV). Lysine release was stimulated 10 to 40% by food deprivation. Furthermore, the addition of leucine, isoleucine and valine to muscle of fasted rats resulted in greater increments of alanine and lysine release than observed with muscle of fed animals. The basal release of glutamine decreased 50% upon fasting. Interestingly, the addition of increasing amounts of the branched chain amino acids stimulated glutamine release from diaphragms of fasted rats, even though they had no effect on its release from muscles of fed animals (Table IV).

Since increased degradation (and hence increased deamination) of branched chain amino acids correlated with greater production of alanine, the branched chain amino acids themselves may donate their amino groups for alanine synthesis. The rate of decarboxylation of [1-14C]leucine, isoleucine, and valine was therefore measured in the same experiment shown in Table IV to determine whether amino groups released upon degradation of the branched chain residues can account for amino groups recovered in alanine. In muscles from fed animals, the amount of branched chain amino acid degradation exceeded the amount of alanine released at both concentrations (Table V). In muscles from fasted rats, both processes increased, but degradation still equaled or exceeded alanine release. However these data do not directly compare the actual rates of alanine synthesis and branched chain amino acid oxidation since the measurements of 14CO2 production do not include the oxidation of nonradioactive amino acids released by protein breakdown and ignore the changes in intracellular specific activity upon addition of labeled branched chain amino acids. In addition, measurements of alanine release include some amino acid derived from protein breakdown and not synthesized de novo. To account for some of these factors, we also compared the increments in 14CO2 production and alanine release when the concentration of the branched chain amino acids in the medium was raised from 0.1 to 0.5 mM (Table V). The increase in moles of the branched...
In the same experiment as shown in Table IV, 14CO2 production was measured from muscles incubated in media containing [1-14C]leucine, isoleucine, and valine at equal specific activities. The amount of branched chain amino acids oxidized was calculated from the 14CO2 produced and the specific activity of the amino acid in the medium. Figures represent the difference in 14CO2 produced and alanine released by diaphragms incubated in media containing 0.5 mM branched chain amino acids and media containing 0.1 mM branched chain amino acids.

<table>
<thead>
<tr>
<th>Branched chain amino acids added</th>
<th>Fed</th>
<th>Pasted</th>
</tr>
</thead>
<tbody>
<tr>
<td>14CO2</td>
<td>nmol/mg</td>
<td>nmol/mg</td>
</tr>
<tr>
<td>0.1</td>
<td>1.1</td>
<td>0.9</td>
</tr>
<tr>
<td>0.5</td>
<td>6.8</td>
<td>1.9</td>
</tr>
<tr>
<td>Difference</td>
<td>5.7</td>
<td>1.0</td>
</tr>
</tbody>
</table>

The amount of branched chain amino acids degraded still exceeded the observed increase in alanine release. Thus the oxidation of the branched chain amino acids is at least sufficient to account for the observed alanine production. More refined experiments will be necessary to determine the exact stoichiometry.

**Synthesis of Alanine from Exogenous Glucose**—To test whether exogenous glucose could provide the carbon chain for the synthesis of alanine, diaphragms from fasted rats were incubated for 2 hours with [U-14C]glucose. Radioactive carbon atoms from glucose were recovered in alanine, glutamate, glutamine, and aspartate, and no other amino acids. Using the specific activity of the glucose initially added we have calculated that 60% of alanine recovered in the medium and the intracellular pool was derived from exogenous glucose (Table VI). Incorporation from labeled glucose could also account for 25% of the aspartate and glutamate recovered and 12% of the glutamine. Addition of unlabeled branched chain amino acids to the medium increased the total radioactivity and specific activity of alanine, but not of any other amino acid. Thus the addition of the branched chain amino acids increased alanine production about 2-fold with at least 69% of the carbon coming from labeled glucose (Table VI). In a similar experiment employing diaphragms from fed rats, we also observed a stimulation by the branched chain amino acids of the incorporation of labeled glucose into alanine. Under basal conditions, 30% of the alanine recovered was derived from labeled glucose. Addition of the branched chain amino acids again increased both the amount of alanine produced and the incorporation of 14C into this amino acid (Table VI).

These estimates of the fraction of alanine derived from exogenous glucose must represent minimal values because they included the unlabeled alanine present initially in the intracellular pool and the amount released directly from protein breakdown. To minimize these problems we also calculated the specific activity of the alanine produced upon addition of branched chain amino acids (Table V). Computed in this way, in diaphragms of fasted rats, 77% of the increased alanine production was derived from [U-14C]glucose. In muscles from fed rats about 60% of the increased alanine was derived from exogenous glucose. These observations, together with the findings that degradation of leucine, isoleucine, and valine alone can account for the increased release of nitrogen in the form of alanine (Tables III through V), indicate that the branched chain amino acids stimulate the de novo synthesis of alanine.

Experiments were also undertaken to examine whether the supply of glucose and insulin affect alanine production by the diaphragm. In diaphragm from fasted rats, glucose alone increased the amount of alanine recovered, and addition of insulin in the presence of glucose further stimulated alanine production (Table VII). Alanine production again did not correlate with net protein turnover. Earlier studies have shown that addition of glucose and insulin have additive effects in promoting protein synthesis and inhibiting protein breakdown under these conditions (12). By contrast insulin by itself appeared to decrease slightly the production of alanine. This result suggests that insulin might be influencing alanine production by promoting transport or metabolism of glucose. In fact these results were restricted to tissues of fasted animals, where supply of carbohydrates may be limiting. In diaphragms of fed animals, insulin in the presence of glucose repeatedly failed to increase alanine production and may have actually inhibited it.

**DISCUSSION**

At physiological concentrations, the branched chain amino acids have a striking ability to increase the production of alanine, glutamate, and lysine by incubated diaphragms (Table I). By contrast, supply of leucine, isoleucine, and valine decreased release of tyrosine and phenylalanine. Since these latter amino acids can neither be synthesized nor degraded by skeletal muscle (12, 15), the branched chain amino acids must have inhibited the net production of free amino acids from muscle protein that occurs in such incubated tissues. Other work in this laboratory...
showed that supply of leucine, isoleucine, and valine reduced tyrosine production both by stimulating protein synthesis in muscle and by inhibiting protein catabolism (12). Therefore the finding of greater amounts of alanine, glutamate, and lysine under the same conditions must indicate that the branched chain amino acids either promote synthesis or reduce the catabolism of alanine, glutamate, and lysine. In fact, the various experiments presented here strongly indicate a stimulation of de novo synthesis of alanine from exogenous glucose.

Of all the amino acids tested in Table III only leucine, isoleucine, and valine appear to be physiologically important sources of amino groups for alanine synthesis. The presence of other amino acids, even at 2 or 5 times their normal plasma concentrations, failed to stimulate alanine production (Table III). This failure is probably related to the inability of the muscle to degrade these other amino acids to CO₂ at a significant rate (8). Other possible nitrogen sources such as ammonia, adenosine, or adenine also failed to stimulate alanine production in muscle (4). Although Ruderman (4, 16) and Garber et al. (17) have reported that other amino acids do stimulate alanine synthesis, these experiments supplied the amino acids to muscle at 25 to 200 times normal plasma levels and assayed alanine by enzymatic methods. At such high concentrations used, various amino acids may be transaminated to a significant extent. In addition, such high concentrations of other amino acids can cause falsely high measurements of alanine in assays using alanine dehydrogenase (18, 19). In either case, the physiological import of such findings would be questionable.

A variety of observations suggest that the amino groups for alanine, glutamate, and possibly lysine are derived from the oxidation of the branched chain amino acids. Addition of the branched chain amino acid increased the total amount of amino groups recovered (specifically by increasing alanine, glutamate, and lysine) (Table III), even though the branched chain residues reduced the net release of amino acids from protein. Furthermore, the degradation of exogenous leucine, isoleucine, and valine alone released sufficient amino groups to account for the increased production of alanine, glutamate, and lysine (Table V). Food deprivation, which increased the oxidation of the branched chain amino acids by muscle, also stimulated the production of alanine 2- to 3-fold (Table V). Interestingly, alanine production in muscles of fasted rats is more sensitive to the addition of the branched chain amino acids (Table IV) than in muscles of fed animals. This difference may result from the lower \( K_m \) for the oxidation of leucine by the muscles of fasted rats (8, 10). Thus, the accelerated degradation of leucine, isoleucine, and valine may be the driving force for the greater alanine synthesis during fasting.

It is unlikely that the branched chain amino acids donate their amino groups directly to pyruvate to form alanine. Although transaminases for leucine, isoleucine, and valine are found in high amounts in cardiac and skeletal muscle (10, 20, 21), at intracellular concentrations of substrates, the rate of transamination with pyruvate is 100-fold less than with \( \alpha \)-ketoglutarate (10). Therefore the most likely mechanism for alanine synthesis in muscle appears to involve transamination with \( \alpha \)-ketoglutarate, whose intramuscular concentration (22) is close to the \( K_m \) of the transaminase (10). The glutamate formed in this way can then be used for synthesis of alanine by glutamic-pyruvic transaminase, which is highly active in muscle (23, 24). This reaction sequence is supported by the finding that the branched chain amino acids always increased the production of glutamate as well as alanine (Tables III and IV). In addition, glutamate has been found to stimulate alanine production by rat diaphragms (4).

Garber et al. (17) have suggested that the carbon skeletons of other amino acids are incorporated directly into alanine, but no direct evidence for this conclusion was presented. The present studies have shown that a major source of the alanine carbon chain is exogenous glucose, which has been previously found to be a precursor for alanine incorporated into muscle protein (25, 26). Evidence for alanine production from exogenous glucose was obtained upon addition of insulin and glucose. These agents reduce the net release of amino acids from skeletal muscle by stimulating their incorporation into protein or by inhibiting protein catabolism (12). The finding in Table VII of greater alanine production in muscles from fasted rats caused by glucose alone or glucose in the presence of insulin must have resulted from the increased synthesis of alanine. These observations suggest that alanine production in such muscles requires an adequate supply of carbohydrate precursors. The further stimulation by insulin presumably reflects its ability to promote the transport and metabolism of glucose (27-29) since the hormone by itself failed to increase alanine production (Table VII). In diaphragms of fed animals in which glucose transport is much more rapid than in those of fasted animals (30), alanine production in the presence of glucose was not stimulated by insulin (Table VII). These observations may indicate that in fed animals the supply of glucose does not limit alanine synthesis or that in the presence of insulin glucose carbon atoms were diverted to glycogen synthesis or to CO₂ rather than to alanine.

Experiments with \([U-\text{14}^\text{C}]\)glucose provided more direct evidence for alanine synthesis from exogenous glucose. Under basal conditions, at least 30% of the alanine recovered from diaphragms of fed rats and 80% of that from tissues of fasted rats contained carbon from the labeled precursor (Table VI). Since the alanine recovered at the end of the experiment also included preformed alanine resulting from net protein breakdown and unlabeled alanine that may have been present in tissue pools, the actual incorporation of glucose carbon atoms is probably greater than these measurements suggest. In fact, when alanine production was stimulated with branched chain amino acids, the incorporation of \(^1\text{C}\) from labeled glucose accounted for about 60% of the increased alanine production in diaphragms of fed
rats and for almost 80% of the increased production in diaphragm of fasted rats.

As discussed above, these measurements of alanine synthesis (Tables V and VI) do not take into account the ability of the branched chain amino acids to inhibit the release of unlabeled alanine by protein breakdown (12). This effect would increase the specific activity of the recovered alanine and therefore would lead to an underestimate of the amount of alanine derived from exogenous glucose. Muscle glycogen may also possibly serve as a precursor for alanine synthesis. In fact, exercise markedly increases the release of alanine from human muscle (31). Under these conditions glycogen appears to be an important precursor of alanine, since in patients lacking muscle phosphorylase, exercise not only fails to increase the release of alanine, but results in net alanine uptake by the muscle (31).

Unlike alanine, glutamine did not increase upon addition of the branched chain amino acids (Table II) to muscle of fed rats. Garber et al. (17) have observed small increases in glutamine production with 10 mM amino acids, although the physiological significance of these findings is questionable. Glutamine synthase has been reported in skeletal muscle (32) and thus glutamate may therefore be a precursor of both glutamine and alanine. However, increasing the glutamate supply by addition of the branched chain amino acids only stimulated the synthesis of alanine (Table II). In the incubated muscles glutamine formation may possibly be limited by lack of additional factors such as ammonia. In fact Ruderman and Lund (4) have observed that high concentration of ammonia stimulated the production of glutamine but not alanine in rat hindquarters.

The branched chain amino acids also increased the production of lysine by muscles of fed (Table III) and fasted (Table IV) rats in a concentration-dependent manner. At present there is no evidence for lysine synthesis in muscle or other mammalian tissues. Possibly, the branched chain amino acids may enhance lysine release by inhibiting its degradation. Radioactive metabolites of lysine have been found in skeletal muscle (33), although lysine does not appear to be degraded to CO2 by the diaphragm (8). In addition, the formation of saccharopine from lysine has been shown in extracts of human heart muscle (34), and recently lysine has been reported to be a precursor for carnitine biosynthesis in rat skeletal muscle (33).

**Branched Chain Amino Acid-Alanine Cycle—Mallette et al.** (14) and Felig et al. (1, 35) independently proposed an “alanine cycle” in which synthesis of alanine by skeletal muscle provides a vehicle for transporting carbon and nitrogen to the liver for gluconeogenesis and urea formation. Although the present experiments have provided direct evidence for alanine production from circulating glucose, they suggest certain modifications of this cycle in light of the apparent coupling between catabolism of the branched chain amino acids and alanine synthesis (Fig. 1). Our formulation (Fig. 1) suggests that release of the branched chain amino acids by liver may be an important determinant of the rate of alanine synthesis by muscle. In the absence of insulin, increased net protein breakdown in liver enhances the supply of leucine, isoleucine, and valine to muscle. It has also been shown that these amino acids unlike most others are not utilized for gluconeogenesis and instead are released by the liver into the blood (14). In fact, increased hepatic production of leucine, isoleucine, and valine has been observed under conditions where net protein breakdown and gluconeogenesis is enhanced (e.g. in perfused livers from diabetic (36), uremic (37) and fasted rats (38), or livers treated with glucagon (38)). At the same time uptake and degradation of the branched chain amino acids provide the sole source of amino groups for alanine synthesis. In fact, the muscles of both diabetic (10, 39) and fasted rats (8, Table V) show an increased capacity to burn leucine, isoleucine, and valine.

This coupling of the metabolism of leucine, isoleucine, and valine with alanine synthesis may help explain alterations of blood alanine and glucose levels in certain human diseases. Tissues from children with maple syrup urine disease are unable to deaminate the branched chain amino acids (40, 41). These patients have plasma alanine levels 3- to 10-fold lower than normal (42) and suffer from hypoglycemic episodes (43-45) although insulin levels are normal (46). The block in catabolism of the branched chain amino acid may have reduced alanine synthesis in muscle and caused the hypoglycemia. In ketotic hypoglycemia (47, 48) children become severely hypoglycemic after only short periods of food deprivation, even though they appear to have a normal gluconeogenic apparatus (47, 49, 50). The hypoglycemic episodes occur concomitantly with severely lowered plasma alanine and can be corrected by alanine infusions (50). In addition, these episodes are characterized by increased concentrations of leucine, isoleucine, and valine (49).

These observations suggest a defect in alanine synthesis which may be caused by an inability to increase the degradation of the branched chain amino acids.

The concurrence of decreased plasma alanine levels and hypoglycemic episodes in these conditions emphasizes the importance of alanine in glucose homeostasis. Yet the alanine cycle as originally proposed does not yield net synthesis of glucose. The metabolism of glucose to alanine, like its metabolism to lactate in the Cori cycle, can contribute to the energy demands of the muscle without any net consumption of glucose carbon. In terms of ATP production the alanine cycle appears more efficient than the Cori cycle, since the conversion of 1 mol of glucose to alanine provides 8 mol of ATP compared to only 2 mol for conversion to lactate (assuming the NADH produced by glycolysis is eventually oxidized by oxidative phosphorylation (91)). Furthermore, formation of alanine by transfer of amino groups to pyruvate from the branched chain amino acids (via sequential transamination as described above) should allow the complete oxidation of leucine, isoleucine, or valine which would yield an additional 42, 43, or 32 ATP per mol, respectively (52). Accordingly, the branched chain amino acid-alanine cycle may provide up to 25 times more ATP than the Cori cycle.

Although skeletal muscle burns primarily glucose and fatty acids, in vivo measurements of oxidation of these substrates do not account for the total O2 consumption of this tissue (10). Using data on alanine release, tyrosine release, and total oxygen consumption of human muscles (10, 53), and the value given above for ATP production, we have calculated that the branched chain amino acid-alanine cycle (Fig. 1) may account for 14% energy supply of these muscles. In addition, preliminary measurements on the incubated rat diaphragm indicate that the
oxidation of the branched chain amino acids may account for 20 to 30% of the muscle’s oxygen consumption. Thus the coupling of branched chain amino acid oxidation to alanine synthesis may serve as an important adaptation to fasting by providing a non-carbohydrate energy source for muscle and thus sparing glucose.

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REFERENCES

Origin and Possible Significance of Alanine Production by Skeletal Muscle
Richard Odessey, Edward A. Khairallah and Alfred L. Goldberg


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