Effects of Intrabrachial Arterial Infusion of Pyruvate on Forearm Tissue Metabolism

INTERRELATIONSHIPS BETWEEN PYRUVATE, LACTATE, AND ALANINE

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ABSTRACT Postabsorptive release of alanine from forearm skeletal muscle is large relative to other amino acids, suggesting new synthesis by transamination of pyruvate. This hypothesis was tested and the pathway quantified in six subjects, each given two 30 min intrabrachial arterial pyruvate infusions. The first (12 μmoles/min) supplied approximately that amount of pyruvate produced endogenously by glycolysis in resting muscle. The second (36 μmoles/min) approximated endogenous pyruvate production by glycolysis during moderate exercise. Changes in balance across forearm tissues of pyruvate, glucose, lactate, and amino acids were measured. The time-course of pyruvate equilibration across forearm muscles was detailed in three additional subjects.

The two infusions increased arterial pyruvate from 64 to 674 and 1776 μmoles/liter respectively. Muscle consumed 72% of the exogenous pyruvate during both infusions. Outputs of lactate and alanine increased, accounting respectively for 30.3 and 6.7% of the pyruvate at the low infusion rate, and 17.1 and 3.8% at the high rate. The remaining pyruvate probably was oxidized. Muscle release of valine, isoleucine, and leucine decreased during the high dose infusion. Additionally, adipose tissue plus skin released more alanine and lactate during the high dose infusion. Other metabolies were unchanged.

Thus, both muscle and adipose tissue plus skin synthesize alanine from pyruvate. Lactate production considerably exceeds that of alanine. In muscle, increased availability of intracellular pyruvate serving as a nitrogen acceptor may facilitate branched chain amino acid oxidation. Muscle consumption of infused pyruvate is rapid, and detailed studies of its equilibration suggest that passage across the muscle cell membrane is rate limiting.

A preliminary report of this work has appeared in abstract form (1).

Dr. Tancredi was recipient of U. S. Public Health Service Special Fellowship 1 FO3 HE 44504.

Received for publication 7 March 1972 and in revised form 3 May 1972.

INTRODUCTION

Previous studies on the human forearm preparation have shown that in overnight-fasted man the concentration of amino acids is higher in venous blood draining skeletal muscle than in arterial blood (2–4). A negative balance, or output, was observed for virtually all the individual amino acids, glutamic acid being the chief exception. The pattern of release was remarkable for the large outputs of alanine and glutamine, each exceeding by at least 2½-fold the output of any of 19 other amino acids. In addition, the free intracellular pools of these two amino acids are known to be large (5, 6).

These observations seem best explained by substantial *de novo* synthesis and release of alanine, glutamine, and perhaps other nonessential amino acids by muscle. Alternative hypotheses to be considered include the preferential accumulation in muscle of these amino acids under postprandial conditions for release postabsorptively, the breakdown of a protein (or proteins) rich in alanine and glutamine, or the selective consumption by muscle of certain amino acids, derived either from the diet or from endogenous protein, in preference to alanine and glutamine.

The present investigation was designed to determine

whether nonessential amino acids are synthesized in the peripheral tissues of intact man, and to quantify this pathway. Our approach has been to provide in excess a naturally occurring nonnitrogenous substrate which can enter cells for conversion to amino acids while maintaining constancy of other parameters likely to influence nitrogen metabolism. Therefore, pyruvate, the anticipated precursor of alanine, was infused directly into the brachial artery of postabsorptive man at rest, and changes in the balance of pyruvate, lactate, glucose, alanine, and other amino acids across forearm tissues were measured.

METHODS

Nine normal males aged 22–30 yr volunteered for studies beginning at 8:30 a.m. after a 14 hr fast. Body weight ranged from 98 to 119% of ideal.[1] With the subject supine a needle was introduced into the brachial artery opposite the direction of blood flow and a catheter threaded through it and into the artery so as to terminate about 1 cm beyond the needle tip. The central catheter was used to sample blood entering the forearm uncontaminated by solutions infused 1 cm downstream through the space between the outer wall of the catheter and the inner wall of the needle. Another catheter was introduced into a large antecubital vein and threaded deep into forearm tissues toward the wrist. Finally, a scalp vein needle was placed in a superficial forearm vein. Blood samples could then be drawn simultaneously from the artery, the deep vein draining mainly forearm muscle, and the superficial vein draining mainly subcutaneous adipose tissue plus skin. Muscle metabolism was estimated from measurements of metabolite arterio-deep venous concentration difference (A-DV),[2] and arterio-superficial venous concentration difference (A-SV) was taken to reflect the metabolism of subcutaneous adipose tissue plus skin. Forearm plasma (and blood) flow was measured at each collection period by a continuous infusion dye-dilution method (7). Changes in metabolite balance due to pyruvate infusion were calculated from changes in arteriovenous concentration difference under conditions of constant blood flow. A sphygmomanometer cuff placed about the wrist was inflated above arterial pressure 5 min before and during blood collections to exclude tissues of the hand from study. The cuff was also inflated during pyruvate infusions. Care was taken to maintain muscles of the arm at rest. Forearm volume between the humeral epicondyles and the sphygmomanometer cuff was measured by water displacement.

Sterile pyrogen-free stock solutions of sodium pyruvate (nominally 145 mM) were prepared periodically during the course of these studies, adjusted to pH 5.8 with a small volume of 0.1 N HCl (less than 0.1 ml/100 ml of stock solution) to minimize pyruvate breakdown, distributed in 10 ml single-dose injection vials, and refrigerated at 4°C for final dilution just before infusion. The stock solutions were discarded within 2½ months. At the time of final dilution the pyruvate concentration of each stock solution was determined in duplicate and no diminution was noted. The final osmolality of injectates made from these solutions was from 224 to 270 milliosmols/kg water.

In six of the nine subjects pyruvate was infused at both a low and a high rate. For the low dose infusion pyruvate stock solution was diluted with an aqueous solution of Evans blue dye to a final pyruvate concentration averaging 112 mmoles/liter and injected into the brachial artery at a constant rate of 0.107 ml/min for 30 min. For the high dose infusion which began approximately 30 min after terminating the low, a solution containing 133 mmoles of pyruvate/liter in Evans blue was injected over 30 min at a constant rate of 0.270 ml/min. Consequently, there was a threefold difference in the rate of pyruvate delivery between the low (12 μmoles/min) and the high (36 μmoles/min) dose infusions. Two sets of arterial and venous blood samples were drawn over a 60 min control period; the derived data were averaged and have been designated 0 time. Subsequent samples were obtained at 25, 30, and 60 min after initiating the low dose infusion. Approximately 5 min after the 60 min collection the high dose infusion was begun and blood samples were obtained 25, 30, and 60 min later.

Quantification of metabolism by the Fick principle requires constancy of metabolite arteriovenous concentration difference (8). To confirm that a new steady state of pyruvate arteriovenous difference was achieved under the conditions of the present experiments, studies to define precisely the time-course of pyruvate arteriovenous concentration difference were performed in three of nine subjects at the high infusion rate only with more frequent sampling during the infusion (3, 6, 9, 12, 15, 20, 25, and 30 min).

Arterial and venous blood was collected in heparinized syringes and 2 ml from each syringe immediately discharged into preweighed tubes containing 6 ml of iced perchloric acid. Lactate was determined in triplicate and pyruvate in duplicate by enzymatic methods (9, 10). A Somogyi-Nelson filtrate of whole blood was also made immediately and glucose determined in triplicate by a modification of the glucose oxidase method (11). Plasma was separated from the remainder of each blood sample by centrifugation at 4°C. Protein-free supernatants, representing a 1:2 dilution of plasma in 10% sulfosalicylic acid, were prepared for amino acid analysis and frozen. Neutral and acidic amino acids were measured within 1 month on an Amino Acid Analyzer (Model 121, Beckman Instruments Inc., Spinco Div., Palo Alto, Calif.). Glutamine and asparagine are not separated by the chromatographic technique employed and, in addition, are known to be gradually lost from protein-free supernatants of plasma principally because of the formation of ninhydrin-negative cyclic compounds. Seven protein-free supernatants of plasma were chromatographed serially over periods of storage at −20°C for up to 50 days, and the average decline in the glutamine-asparagine peak was 2.1±0.82 (SEM) μmoles/liter per day (range 1.08–7.62). Because of this rapid and variable rate of decay, results for glutamine-asparagine are not reported. A small portion of the glutamine is lost by deamidation to glutamic acid, which itself is stable (12). Glutamic acid concentration in these same seven protein-free supernatants increased slowly, at a rate of 0.6±0.08 μmoles/liter per day. To minimize errors in the calculated arteriovenous concentration differences for glutamic acid, supernatants from corresponding arterial and venous plasma samples were always chromatographed on the same day. Control values for glutamate A-DV in the present study were the same as those reported previously when samples were analyzed within 3 days (4). A two-tailed Student's *t* test was used to determine the significance of changes from 0 time in

[1] From the Metropolitan Life Insurance tables, 1959.

[2] *Abbreviations used in this paper:* A-DV, arterio-deep venous concentration difference; A-SV, arterio-superficial venous concentration difference; MTT, mean transit time.

metabolite arteriovenous concentration difference under conditions of stable flow, each subject serving as his own control (13). All arteriovenous differences were calculated from direct measurements of both arterial and venous metabolite concentration except in the case of pyruvate during periods of infusion. For pyruvate, its arterial concentration distal to the site of pyruvate infusion, $[P]_A$, was calculated as follows:

$$[P]_A = [P]_E + [P]_{A_{Recirc}},$$

where $[P]_E$ is the enrichment of arterial pyruvate attributable to the infusion and $[P]_{A_{Recirc}}$ is the pyruvate content of arterial blood proximal to the site of infusion and recirculating into the forearm.

The pyruvate enrichment of arterial blood, $[P]_E$, attributable to pyruvate infusion was calculated as shown below:

$$[P]_E = \frac{[P]_I \cdot F_I}{F_B},$$

where $[P]_I$ is the pyruvate concentration in the infusate, F_I the flow of infusate, and F_B the forearm blood flow.

RESULTS

Base line measurements (Table I). Postabsorptive consumption of glucose by deep and superficial tissues was readily demonstrable (Table I). Release of lactate from muscle was of borderline significance ($P < 0.1$); however, net output was observed in eight of the nine subjects and has been reported by other investigators (14, 15). Pyruvate balance across muscle was not different from zero. Muscle released most amino acids, with net uptakes demonstrable for glutamic acid and cystine. As noted previously, alanine output was large (2, 3).

Superficial tissues released both lactate and pyruvate. Amino acid arteriovenous differences were generally less negative across superficial tissues than deep, although significant outputs of alanine, threonine, proline, glycine, methionine, tyrosine, and phenylalanine were observed. Alanine output was at least 4½-fold greater than that of any other amino acid measured. Glutamic acid and cystine were taken up. The pattern of amino acid balance across superficial tissues differed from that of muscle, since the branched chain amino acids were not released by superficial tissues. Forearm plasma flow was 1.6±0.15 ml/min per 100 ml forearm.

Effects of pyruvate infused at low and high rates on pyruvate consumption by forearm tissues (Fig. 1, Table II). After control blood collections were completed, pyruvate was infused intra-arterially for 30 min at a rate of 12 μmoles/min in six subjects. Sets of arterial and venous blood samples were drawn 25, 30, and 60 min after starting the infusion. Approximately 5 min after the 60 min samples were drawn a second 30 min pyruvate infusion was begun at 36 μmoles/min, with blood again sampled at 25, 30, and 60 min. No discomfort was reported. Forearm plasma flow tripled in one subject at the 25 and 30 min periods during the low dose infusion, but then fell to base line values and subsequently was stable. However, because of uncertainties both in the measurement of flow and the interpretation of changing arteriovenous differences under nonsteady-state conditions, all data from this subject at the 25 and 30 min periods of the low dose infusion were discarded. Among the remaining five subjects flow during the low dose infusions fell slightly (significant at 25 min only) but was unchanged thereafter when compared with 0 time (Fig. 1). There was no change in the pyruvate concentration of recirculating arterial blood sampled proximal to the site of infusion as shown in Fig. 1.

At 25 min during the low dose infusion the calculated enrichment of pyruvate in arterial blood, $[P]_E$, was 674 μmoles/liter. Concomitantly, A-DV rose 485 μmoles/liter, from +11 to +496 μmoles/liter, reflecting the fractional consumption by muscle of 0.72 of the infused pyruvate (Table II). A-SV increased by 366 μmoles/liter (from −21 to +345 μmoles/liter) reflecting the fractional consumption of 0.54 of the infused pyruvate. The increase in pyruvate A-DV at 30 min was slightly less than at 25 min (445 μmoles/liter), but the calculated $[P]_E$ also was somewhat lower (630 μmoles/liter) and the fractional consumption unchanged. Fractional consumption by superficial tissues at 30 min was 0.53. A-DV and A-SV returned to levels not different from

TABLE I

Postabsorptive Balance of Glucose, Lactate, Pyruvate, and Amino Acids across Deep and Superficial Tissues in Nine Subjects

	Muscle (A-DV)	Subcutaneous adipose tissue plus skin (A-SV)
	μmoles/liter	*μmoles/liter*
Glucose	+240±32.5* (SEM)	+293±52.0*
Lactate	−92±50.0	−190±33.1*
Pyruvate	+6±6.8	−18±2.8*
Alanine	−88±5.9*	−60±7.7*
Threonine	−18±1.6*	−9±3.0*
Serine	0±1.0	+1±1.6
Proline	−27±5.9*	−14±5.7*
Glutamate	+44±6.0*	+41±6.8*
Citrulline	+2±0.9	+2±1.0
Glycine	−26±2.5*	−8±2.6*
α-Aminobutyrate	0±0.8	+1±1.0
Valine	−7±2.6*	+2±1.8
½-Cystine	+15±1.5*	+10±2.5*
Methionine	−3±0.8*	−2±0.6*
Isoleucine	−5±1.4*	0±1.0
Leucine	−11±3.5*	0±1.7
Tyrosine	−6±0.4*	−2±0.8*
Phenylalanine	−6±0.8*	−3±0.6*

* Value is significantly different from 0 ($P < 0.05$ to < 0.001).

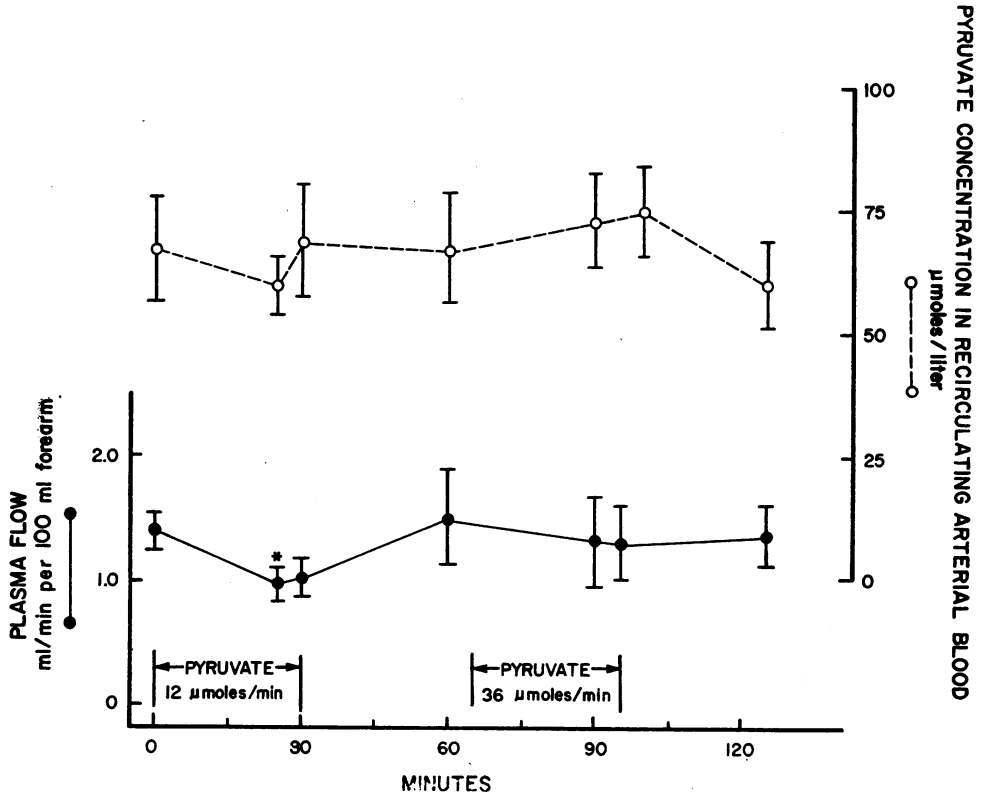

FIGURE 1 Stability of forearm plasma flow and recirculating arterial pyruvate concentration in six subjects given two 30 min pyruvate infusions at low and high rates. Values are means ±SEM. The asterisk indicates a point that is significantly different from 0 time.

base line by 60 min, just before the high dose infusion was begun.

During the high dose pyruvate infusion arterial pyruvate was enriched by 1779 μmoles/liter at 25 min and 1662 at 30 min. The associated increases from 0 time in pyruvate A-DV were 1282 and 1190 μmoles/liter respectively. The fractional consumption of infused pyruvate by muscle was 0.72 at both time intervals and was identical with that observed during the low dose infusion. Fractional consumption by superficial tissues was

TABLE II

*Effect of Pyruvate Infused for 30 min at Low (12 μmoles/min) and High (36 μmoles/min) Rates on Pyruvate Balance across Forearm Tissues in Six Subjects**

	Base line	Min after starting low dose infusion			Min after starting high dose infusion		
	0	+25	+30	+60	+25	+30	+60
Muscle							
A-DV pyruvate, *μmoles/liter*‡	+11±9.3	+496±34	+456±56	−5±12.5	+1293±221	+1201±164	−39±22.3
Fractional consumption of infused pyruvate§		0.72±0.038	0.71±0.064		0.72±0.062	0.72±0.042	
Adipose tissue plus skin							
A-SV pyruvate, *μmoles/liter*‡	−21±2.2	+345±62	+314±87	−28±13.2	+835±187	+804±173	−103±23
Fractional consumption of infused pyruvate§		0.54±0.081	0.53±0.157		0.48±0.105	0.50±0.091	

* N = 5 at +25 and +30 min of the low dose infusion.

‡ During pyruvate infusion, the total pyruvate concentration in arterial blood supplying forearm tissues, $[P]_A$, was calculated as described under Methods. Deep and superficial venous pyruvate concentrations were measured directly and subtracted from $[P]_A$.

§ The fractional consumption of infused pyruvate by muscle was calculated as $\Delta(A-DV)/[P]_E$ and by adipose tissue plus skin as $\Delta(A-SV)/[P]_E$.

somewhat less. Pyruvate washout from deep tissues was virtually complete at 60 min, but washout from superficial tissues was slower, with A-SV significantly more negative at 60 than at 0 min (−103 vs. −21 μmoles/liter, $P < 0.025$).

Validation of new steady-state conditions for muscle pyruvate consumption during pyruvate infusion (Fig. 2). When arterial pyruvate concentration is acutely raised and maintained at a new level, its concentration in venous blood will rise more slowly to a plateau. A stable venous concentration is reached when equilibration with extravascular spaces is complete. Pyruvate consumption by forearm tissues can be estimated only after pyruvate arteriovenous difference has become constant (8). Before this time pyruvate consumption is overestimated to the extent that a portion leaving the vascular space is not metabolized but serves to elevate the pyruvate concentration in extravascular compartments to a new level. In the studies described above, there was no change in fractional pyruvate consumption between 25 and 30 min during infusions at both rates (Table II). Thus, venous pyruvate concentration appeared to stabilize before 25 min and it seemed that a new steady state of increased pyruvate consumption was attained. This conclusion was further substantiated by studies performed in three additional subjects where deep venous pyruvate concentration was measured and fractional consumption calculated at frequent intervals during a single 30 min pyruvate infusion (36 μmoles/min). The pyruvate concentration of deep venous blood rose to a plateau by 15 min, but the increment was only 28% of the calculated pyruvate enrichment in arterial blood, $[P]_B$. Consequently, fractional pyruvate consumption by muscle was 0.72 as illustrated in Fig. 2, and constant after 15 min of infusion. This value for muscle pyruvate consumption is in good agreement with values given in Table II and verifies the attainment of new steady-state conditions. Mean forearm plasma flow in these three subjects measured at 0, 25, and 30 min was 2.0, 2.0, and 1.9 ml/min per 100 ml forearm respectively. Unfortunately, rapid sampling of superficial venous blood in these three subjects was technically difficult and data comparable to that for the deep system were not obtained.

Balances of glucose, alanine, lactate, and other amino acids during pyruvate infusion (Tables III, IV). Muscle balance of glucose was unchanged throughout both py-

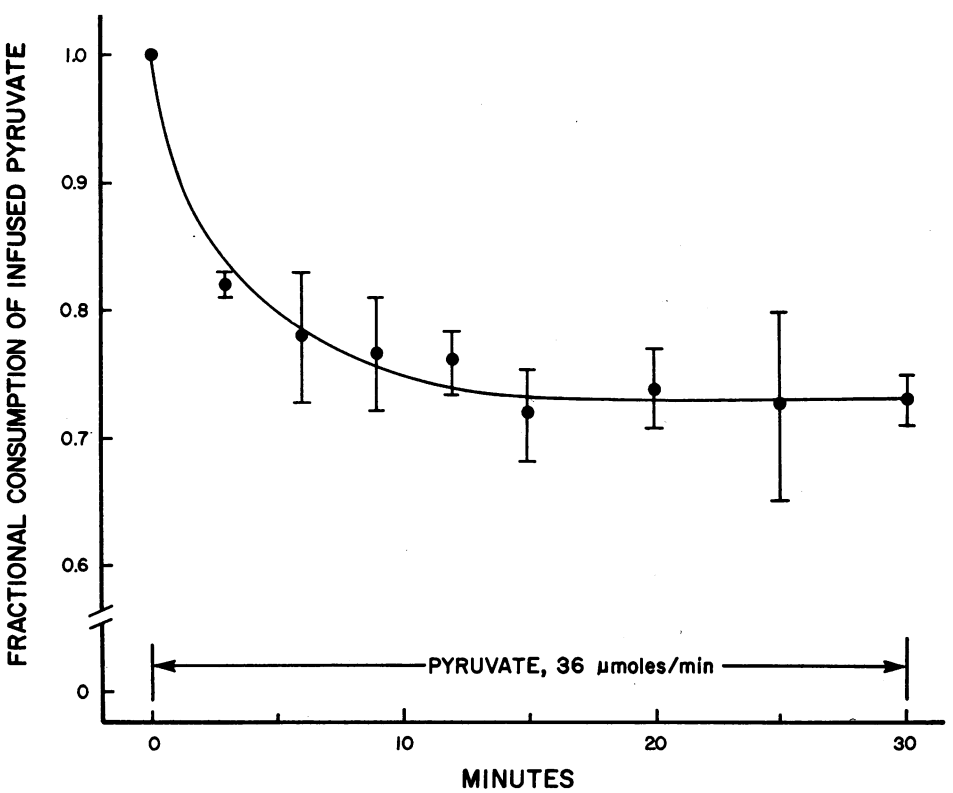

FIGURE 2 Fractional consumption of infused pyruvate by forearm muscle in three subjects. To calculate fractional consumption during pyruvate infusion the change in deep venous pyruvate was subtracted from the enrichment in arterial pyruvate ($[P]_B - \Delta[P]_{DV}$) and divided by $[P]_B$. Values given are means ±SEM.

TABLE III

Balances of Glucose, Alanine, and Lactate across Muscle (A-DV) and Adipose Tissue Plus Skin

	Arteriovenous concentration difference			
	Base line 0	Min after starting low dose infusion +25	+30	+60
	μmoles/liter	*μmoles/liter*	*μmoles/liter*	*μmoles/liter*
Muscle (A–DV)				
Glucose	+233±39.4 (SEM)	+289±78.3	+261±16.1	+189±21.7
Alanine	−86.3±7.17	−113±7.2 (< 0.005)§	−111±8.9 (< 0.02)	−90.9±7.42
Lactate	−56.8±85.60	−190±65.7 (< 0.01)	−239±64.3 (< 0.02)	−176±72.6 (< 0.025)
Adipose tissue plus skin (A–SV)				
Glucose	+300±71.7	+283±77.2	+294±39.4	+189±49.4
Alanine	−52.9±9.90	−61.0±10.37	−68.8±9.25	−59.2±8.18
Lactate	−184±60.8	−213±42.0	−234±42.7	−241±42.6 (< 0.02)

* $N = 5$ for all values at +25 and +30 min during the low dose infusion. Data from one subject were excluded at these time periods because of a transient large increase in flow.

‡ Arteriovenous differences at +25 and +30 min for each subject were averaged and the 0 time (base line) value subtracted to calculate the average change in glucose and alanine arteriovenous difference during each infusion. Immediately before starting the high dose infusion, A–DV and A–SV for lactate were −176±72.6 and −241±42.6 μmoles/liter respectively, significantly more negative than at 0 time. Consequently, these values were used as the basis against which to calculate changes in lactate balance during the high dose infusion.

§ Significance of the change from 0 time (paired *t* test). For lactate, during the high dose infusion, comparison is made with the +60 min value of the low dose infusion.

‖ Sample not obtained in one subject ($N = 5$).

ruvate infusions (Table III), but A-DV for alanine and lactate became significantly more negative. Alanine A-DV achieved a new plateau at both rates of infusion, indicating new steady-state conditions of increased net release. Changes at 25 and 30 min for all subjects during the low dose infusion were averaged and amounted to −29.3 μmoles/liter (Table III). During the high dose infusion the change from base line (0 time) in alanine A-DV at 25 and 30 min averaged −46.2 μmoles/liter. With regard to lactate, a new steady state of increased net release from muscle was approximated. Although lactate A-DV at 30 min tended to be more negative than at 25 min during the low dose infusions, the reverse was true in the high dose studies. However, at neither infusion rate were 25 min values significantly different from those at 30 min. During the low dose infusion the average of changes in lactate A-DV at 25 and 30 min was −158 μmoles/liter. Net lactate release was still significantly greater than base line at the 60 min period of the low dose studies, just before the high dose infusion was begun (lactate A-DV −176±72.6 vs. −56.8±85.60 μmoles/liter, $P < 0.025$ by paired *t* test). Hence, in the case of lactate this 60 min value was used as a new base line against which to assess subsequent changes in balance. The average of changes in lactate A-DV at 25 and 30 min was −232 μmoles/liter during the high dose infusion.

Glucose balance across superficial tissues was unaffected by the pyruvate infusions; a significant increase in alanine release from subcutaneous adipose tissue plus skin was observed during the high dose infusion. Lactate A-SV was slightly but significantly more negative at 60 min of the low dose infusion, and against this new base line a further increase in net lactate release from superficial tissues was observed during the high dose studies, the change in A-SV averaging −94.5 μmoles/liter of superficial tissue blood flow.

There was no demonstrable effect of pyruvate during the low dose pyruvate infusion (12 μmoles/min) on the A-DV for amino acids other than alanine. This was not the case, however, for the infusion at 36 μmoles/min as shown in Table IV. In addition to *increasing* release of alanine, pyruvate infusion at 36 μmoles/min *diminished* the output of the branched chain amino acids leucine and isoleucine at all time intervals, A-DV becoming less negative. At the 60 min period net output of valine had

(*A–SV*) *during 30-min Infusions of Pyruvate at Low and High Rates in Six Subjects**

Arteriovenous concentration difference			Average change in A–V during pyruvate infusion‡	
Min after starting high dose infusion				
+25	+30	+60	Low dose	High dose
μmoles/liter	*μmoles/liter*	*μmoles/*liter	*μmoles/liter*	*μmoles/liter*
+329±63.3	+228±59.4	+250±47.2	+50.6±25.4	+45.5±34.6
−130±18.6	−135±16.3	−135±18.4	−29.3±4.1	−46.2±9.7
(< 0.025)	(< 0.02)	(< 0.01)	(< 0.001)	(< 0.001)
−420±58.2	−396±58.5	−245±63.7	−158±19.5	−232±27.6
(< 0.005)	(< 0.005)	(< 0.005)	(< 0.001)	(< 0.001)
+344±50.0	+367±52.2‖	+300±50.0	+17.9±26.1	+40.9±42.2
−80.3±7.77	−83.9±9.34‖	−77.3±9.15	−14.3±8.7	−24.6±10.1
				(< 0.05)
−335±48.7	−336±79.4	−303±34.8	−39.6±18.6	−94.5±17.8
(< 0.005)		(< 0.005)		(< 0.001)

TABLE IV

Effect of Pyruvate Infused at a High Rate on Muscle Amino Acid Balance (A–DV) in Six Subjects

Amino acid	Base line 0	Min after starting pyruvate at 36 μmoles/min for 30 min		
		+25	+30	+60
	μmoles/liter	*μmoles/liter*	*μmoles/liter*	*μmoles/liter*
Threonine	−18±1.5 (SEM)	−15±4.8	−16±2.9	−18±4.6
Serine	0±0.9	+5±1.5 (< 0.05)*	+5±2.1	+3±2.0
Proline	−36±6.2	−27±8.6	−20±5.7	−20±7.3
Glutamate	+42±7.7	+49±7.9	+48±8.0	+47±6.6
Citrulline	+1±1.0	+4±1.2	0±2.7	0±1.7
Glycine	−25±3.4	−24±6.1	−25±5.3	−27±7.3
Alanine	−86±8.4	−130±18.6 (< 0.025)	−135±16.3 (< 0.02)	−135±18.4 (< 0.01)
α-Aminobutyrate	0±1.2	0±1.6	−1±1.4	0±1.3
Valine	−7±2.3	−6±5.0	−8±2.2	+1±2.7 (< 0.025)
½-Cystine	+14±1.6	+21±3.6	+13±5.4	+16±3.9
Methionine	−3±0.9	−5±0.8	−5±0.8	−4±0.8
Isoleucine	−6±1.3	−1±1.6 (< 0.02)	−3±1.0 (< 0.05)	−1±1.3 (< 0.005)
Leucine	−14±4.5	−7±4.0 (< 0.02)	−8±4.7 (< 0.005)	−6±3.9 (<0.01)
Tyrosine	−6±0.5	−7±1.2	−8±1.2	−6±1.3
Phenylalanine	−6±1.1	−8±1.6	−10±1.8 (< 0.025)	−7±1.3

* Value in parenthesis indicates significance of the change from 0 time (paired *t* test).

also decreased. Significant changes in balance were also noted at single time periods for serine (more positive) and phenylalanine (more negative). No changes in A-SV for amino acids other than alanine were observed at either rate of pyruvate infusion.

Estimated degradation of infused pyruvate within the forearm vasculature (Table V). Since blood normally contains lactic dehydrogenase, some of the infused pyruvate could be converted to lactate within the forearm vasculature. As a consequence tissue pyruvate consumption would be overestimated. This error may be assessed from a knowledge of the residence time for pyruvate in the forearm vasculature and the activity of blood lactic dehydrogenase. The average time spent by a pyruvate molecule in forearm vessels, the mean transit time (MTT), may be expressed:

$$MTT = \frac{V}{F},$$

where V is the volume of the vascular bed (about 8% of the forearm volume) and F the blood flow. The MTT was calculated for each subject using the average of all flow determinations. For the six subjects who received both pyruvate infusions the MTT was 4.1±0.31 min.

To determine lactic dehydrogenase activity, sufficient exogenous pyruvate was added to five samples of shed blood to approximate the arterial levels seen during pyruvate infusion. Pyruvate concentration was then determined repeatedly over a 20 min incubation at 37°C (Table V). The decrease in pyruvate concentration appeared to be linear with time and averaged 6.4±1.69 (SEM) μmoles/min per liter of blood. Consequently, in the infusion studies an estimated 26 μmoles of pyruvate/liter (4.1 min × 6.4 μmoles/min per liter) would be lost in transit through forearm vessels. This could account for no more than 8% of the observed pyruvate arteriovenous concentration differences during the low dose infusion, and considerably less during the high. This small source of error has been neglected in the calculations of tissue pyruvate consumption shown in Table II. The generation of a like amount of lactate added to blood passing through the forearm is of some importance and has been considered in estimates of muscle lactate production from infused pyruvate discussed below. The alanine content of blood during these 20 min in vitro incubations was unchanged.

TABLE V

*Estimated Lactic Dehydrogenase Activity in Five Samples of Blood Incubated at 37°C for 20 min In Vitro**

Blood sample	Initial [P]‡	Rate of pyruvate loss§	No. of observations
	μmoles/liter	*μmoles/liter per min*	
1	954	−12.4	4
2	1751	−4.6	7
3	1703	−5.5	7
4	1648	−7.1	6
5	1178	−2.3	4

* The linear regression of [P] against time was calculated for each sample, and the coefficient of correlation was highly significant.

‡ y intercept (b) of the calculated linear regression $y = mx + b$.

§ The slope (m) of the linear regression.

DISCUSSION

In postabsorptive man a variety of extrahepatic tissues release large amounts of alanine relative to other amino acids. Disproportionate release of alanine from skeletal muscle (2, 3), kidney (16), myocardium (17), and tissues of the gastrointestinal tract drained by the portal vein (18) has previously been documented by organ input-output techniques, and to this list adipose tissue plus skin can now be added. Taken together, these observations suggest that *de novo* alanine synthesis by transamination of pyruvate is common to many tissues. If this hypothesis is correct skeletal muscle, by virtue of its large mass, is the major source of newly synthesized alanine.

Felig and Wahren, in studies of amino acid balance across the leg during bicycle ergometer exercise, have recently provided further evidence for alanine synthesis by peripheral tissues (18). They noted an increase in alanine release from the leg during exercise leading to a rise in systemic (arterial) alanine concentration. Arterial pyruvate concentration also increased, and from the parallel rise in these two metabolites it was inferred that the extra alanine released was newly synthesized from pyruvate generated in leg muscles by the acceleration of glucose uptake and glycolysis. Beyond altering pyruvate availability, however, exercise is also known to affect muscle amino acid transport (19, 20) and protein turnover (20–22). A portion of the additional alanine released could originate from alterations of these processes as well, and consequently, the quantitative importance of the transamination pathway cannot be established from their experiments. Exchange of pyruvate and alanine carbon has been shown conclusively in vitro, where labeling of alanine residues in rat diaphragm protein occurs after incubation with pyruvate-^{14}C (23).

In the present investigation arterial pyruvate concentration was elevated as a single experimental variable, and pyruvate uptake by forearm tissues and conversion to lactate, alanine, and other products quantified under strict steady-state conditions. Results for muscle are summarized in Table VI. The increases

in muscle pyruvate A-DV of + 439 μmoles/liter during the low dose infusion, and + 1211 μmoles/liter during the high are large relative to the normal resting state when pyruvate A-DV is essentially zero; yet, the increase in pyruvate consumption reflected by these values must be within the physiologic range for the following reasons. The basal glucose uptake of 240 μmoles/liter of muscle blood flow observed in the present experiments (Table I) would, in the absence of net glycogen synthesis, generate 480 μmoles of pyruvate/liter, an amount equivalent to the increment in pyruvate consumption of + 439 μmoles/liter of muscle blood flow during the low dose infusion. Hence, pyruvate consumption may have been twice the basal rate. Vigorous exercise is known to increase glucose uptake 10–12-fold (24–26) while glycogen stores are undergoing depletion (27). Since concomitant pyruvate release is trivial (24), muscle pyruvate consumption during exercise must greatly exceed that seen during the high dose infusion.

Alanine was the only amino acid whose output increased in association with increased pyruvate consumption. At the low infusion rate 6.7% of the additional pyruvate consumed by forearm muscle appeared as new alanine (Table VI). Another 30.3% appeared as lactate. Most pyruvate (63.0%) could not be accounted for by either product, and presumably underwent oxidative decarboxylation. At the high infusion rate alanine and lactate outputs both increased over the low to precisely the same extent, 57%, consistent with increased availability of a common precursor, namely intracellular pyruvate. The increase in alanine and lactate production of 57% was surprisingly small in view of the 300% increase in arterial (and venous) pyruvate concentration and pyruvate consumption. This can be expected if the rise in intracellular pyruvate concentration were considerably less than threefold, a possibility supported independently by the surprisingly rapid equilibration of infused pyruvate across forearm muscle (approximately 15 min). Equilibration in this period of time indicates an apparent volume of distribution for pyruvate about the size of the extracellular fluid space. When pyruvate consumption, presumably by the pyruvate dehydrogenase system, is rapid relative to diffusion across the cell membrane, the rise in intracellular concentration would be limited, and the infused pyruvate would appear to distribute in a volume near that of the extracellular fluid space.[3] The fixed fraction of exogenous pyruvate metabolized by muscle (0.72) over a wide range of arterial pyruvate concentrations is also compatible with diffusion limited consumption. In studies of perfused heart (28, 29) and diaphragm incubated in vitro (30) intracellular concentrations of pyruvate well below the perfusate or medium have been noted after enrichment with exogenous pyruvate. Oxygen uptake by resting forearm tissues has been quantified previously (14) and, if other oxidizable substrates are replaced by pyruvate, basal oxygen consumption is adequate to account for the postulated increment in the pyruvate dehydrogenase pathway at both infusion rates.

Despite its primacy among amino acids as a gluconeogenic substrate in postabsorptive man (31), alanine accounts for less than 10% of glucose released by the liver (32, 33). Moreover, to the extent that pyruvate

TABLE VI

*Metabolism of Infused Pyruvate by Muscle**

	Low dose infusion	High dose infusion
Change in A–DV, *μmoles/liter*		
Pyruvate‡	+439	+1211
Alanine	−29.3	−46.2
Lactate§	−133	−207
Per cent conversion of additional pyruvate consumed to:		
Alanine	6.7	3.8
Lactate	30.3	17.1
"Other products"	63.0	79.1

* Values given derive from data in Tables II, III, and V.

‡ 26 μmoles/liter was subtracted from the average increase in pyruvate A–DV (mean of changes from 0 in A–DV at 25 and 30 min, Table II) during each infusion period to correct for the estimated conversion of pyruvate to lactate within the circulation (Table V).

§ 26 μmoles/liter was subtracted from the change in lactate A–DV (Table III) to correct for lactate production within the circulation.

[3] For nonmetabolized indicators, the mean transit time, MTT, through the forearm is equal to the volume of distribution, V, divided by forearm flow (plasma or blood flow depending on whether the substance is distributed in plasma or whole blood). If a substance distributed in whole blood is confined to the forearm extracellular space (20% of forearm volume) then V = 20 ml/100 ml forearm volume, blood flow (our data) is 2.6 ml/min per 100 ml forearm and MTT = 20/2.6 = 7.7 min. Similarly, a freely diffusible substance distributing in the total water space of the forearm (75 ml/100 ml forearm) would have a MTT of 28.8 min. Assuming complete instantaneous mixing during constant rate intra-arterial infusion, one would expect to see an apparent plateau in venous concentration within two to three MTTs, accepting a measurement error of < 10%. Therefore, an extravascular indicator would appear to reach a plateau within 15–23 min, whereas it would require more than 58 min for a substance distributed in the total forearm water space. In the case of a substance which is partially metabolized, only the nonmetabolized molecules contribute to the measured venous concentration. If all of the molecules which enter the cells are rapidly metabolized, then the apparent volume of distribution for nonmetabolized molecules will approximate the extracellular fluid volume.

transaminated in peripheral tissues is itself a product of glycolysis the resulting alanine provides no new carbon for the generation of carbohydrate. Therefore, the role of alanine in glucose homeostasis under post-absorptive conditions probably is small. The importance of alanine synthesis may relate instead to amino acid oxidation with alanine serving as a vehicle for the transfer of nitrogen in nontoxic form from muscle to liver. Muscle can generate ammonia (34); however, oxidation of amino acids ordinarily begins with loss of the amino group by transamination to an α-keto acid such as pyruvate. Terminal oxidation of amino acids by muscle has been demonstrated in vitro (35–37). Valine, leucine, and isoleucine are of special interest because hepatic uptake is small (38–40) and extrahepatic tissues are thought to be the major site of oxidation (41). In the present investigation decreased release of branched chain amino acids was associated with increased alanine release during the high dose pyruvate infusion. This observation is consistent with the view that branched chain amino acid oxidation is enhanced by pyruvate surfeit, although increased consumption of these amino acids by some other pathway cannot be excluded.

Definite changes in alanine and lactate production by superficial tissues were not demonstrable during the low dose infusion. Production of both increased during the high, lactate more than alanine, indicating a similarity to deep tissues in this regard and confirming the suggestion, based on the profile of amino acids released basally, that *de novo* synthesis of alanine from pyruvate is a pathway of potential importance in extrahepatic tissues other than muscle.

ACKNOWLEDGMENTS

The authors wish to acknowledge the technical assistance of Mrs. Lyle Myerberg and Miss Louise Margolet, and the secretarial assistance of Mrs. Kay Zittle.

This work was supported by U. S. Public Health Service Research Grants AM-14754, RR-00035, and AM-05136.

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