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[U-^{14}C]-GLUCOSE, -ALANINE, AND -LEUCINE OXIDATION IN RATS AT REST AND TWO INTENSITIES OF RUNNING

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[U-^{14}C]-GLUCOSE, -ALANINE, AND -LEUCINE OXIDATION IN RATS AT REST AND TWO INTENSITIES OF RUNNING

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Running Head: Amino Acid Oxidation During Exercise

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ABSTRACT

Using rats we investigated the influence of metabolic rate (MR) on the oxidations of glucose (glc), alanine (ala), and leucine (leu) *in vivo*. Evolution of $^{14}CO_2$ was assessed for 2 h following injection of 5 μCi of [U-^{14}C]-glc, -ala, or -leu during rest, during running at 14.3 m/min on a 1% grade, or during running at 28.7 m/min on a 15% grade. Following [U-^{14}C]-glc injection, the rate and magnitude of $^{14}CO_2$ evolution were relatively low at rest and increased proportionally to MR. Recoveries of infused ^{14}C as $^{14}CO_2$ were 42, 67 and 87% at the three MR, respectively. Evolution of $^{14}CO_2$ following [U-^{14}C]-ala injection appeared faster and to a greater extent during exercise than rest, but no difference appeared between the two exercise intensities. Recoveries were 55, 71 and 74%, respectively. The rate and magnitude of $^{14}CO_2$ evolution following [U-^{14}C] -leu injection were proportional to MR, but quantitatively less than following glc or ala injection. Recoveries were 18, 36 and 45%, respectively. We conclude that the metabolism of selected amino acids is integrally linked to the energy transductions contributing to the carbon flow sustaining exercise.

Key Words: small animals, exertion, metabolism, energetics, skeletal muscle, rodent, amino acid, branched chain amino acid, exercise, transamination, deamination, lactate.

INTRODUCTION

Traditional views hold that when caloric supply is adequate, proteins and amino acids contribute insignificantly to the carbon flow sustaining physical exercise (7). These views are based largely upon early studies which observed insignificant alterations in urinary nitrogen excretion to be associated with muscular exercise in humans (17). More recent and tightly controlled studies (25) have also indicated only small and transitory effects of the initiation of training on nitrogen balance. It appears, therefore, that the deamination of amino acids does not contribute significantly to the substrate pool for muscular work.

Several lines of evidence are consistent with the hypothesis that transaminations and other processes are integrally involved with the energy flux during exercise. It is known, for instance, that transaminase activity is high in skeletal muscle (10,30,46). Moreover, Molé, et al. (38) have demonstrated skeletal muscle glutamate-pyruvate transaminase (GPT) activity to increase 85% in response to endurance training in rats. Further, Lowenstein (33) has attributed the formation of ammonia in skeletal muscle to the purine nucleotide cycle (PNC) in which AMP is deaminated to IMP, releasing free ammonia. Potentially, this ammonia can be a nitrogen donor for the formation of glutamate from alpha keto-glutarate (α-kG) and for glutamine formation from glutamate. Glutamine as well as alanine production in rat hemi-corpus preparations has been reported by Ruderman and Bergers (43).

In an extension of earlier studies by Wahren, et al. (47) on amino acid catabolism in humans during starvation, Ahlberg, et al. (4) have demonstrated quantitatively significant participation of the "glucose-alanine cycle" (18)

in the metabolic adjustments to prolonged exercise. Their data indicate the output of alanine by working skeletal muscle to be equivalent to the uptake of branched chain amino acids (BCAA).

Several groups of investigators (10,11,36,27) have independently observed that isolated rat skeletal muscle preparations actively oxidize BCAA, even in the presence of alternative substrates. On the basis of these and related findings, Odessey, et al. (41) have proposed a "branched-chain-amino acid-alanine cycle" in which the BCAA act as nitrogen donors for the formation of alanine from pyruvate. Under this scheme, the BCAA are initially transaminated to form glutamate with the non-nitrogenous residue ultimately forming acetyl or succinyl CoA. In the GPT reaction mentioned earlier, glutamate and pyruvate form alanine and α-KG. In addition to being a tri-carboxylic acid (TCA) cycle intermediate, α-KG is involved in initial BCAA transamination.

Preliminary studies from this laboratory using rats (9) have indicated significant incorporation of ^{14}C from injected [U-^{14}C]-glucose into muscle and blood pools of alanine, glutamine, glutamate, and aspartate. Those observations and previous findings of others suggested to us that the metabolism of particular amino acids could be integrally linked to the energy transductions contributing to the carbon flow sustaining exercise. To test this hypothesis, we decided to study the oxidations of glucose, alanine and leucine during rest and two intensities of running in laboratory rats.

METHODS

Female Wistar rats (Simonson Labs, Gilroy, California) were obtained at 36 days of age, housed individually, provided water and feed (Feedstuffs, Inc., San Francisco, California) ad libitum, and maintained on a 12h/12h,

6:00 AM/6:00 PM, light/dark schedule. The rats trained five days per week for ten weeks until they could run continuously at a speed of 28.7 m/min on a 15% grade for 120 min. Animals were maintained at this level for one week during which time they were also habituated to treadmill exercise in a metabolic chamber (48). Physical training was not used in this study to describe differences between trained and sedentary animals, but rather to allow animals to exercise consistently and sufficiently long for data collection. Eighteen proven runners were assigned on the basis of body weight to one of three substrate injection groups: glucose (glc), alanine, (ala), or leucine (leu). These groups weighed 237.5 $\pm$ 8.0, 236.3 $\pm$ 8.1, 237.5 $\pm$ 9.9 g ($\bar{X} \pm \bar{S}x$), respectively. Within each group there were three metabolic conditions: rest, treadmill running at 14.3 m/min on a 1% grade, and treadmill running at 28.7 m/min on a 15% grade. The exercises used were judged by our experience with these and previous rats to represent easy exercise and the most difficult exercise animals could negotiate for the 2 h period required for $^{14}CO_2$ collection. Over the course of three weeks of testing, a given rat performed at all three metabolic conditions within a given substrate group, each experiment being at least five days apart. Training was maintained 3 times/week for 1.5 h, 28.7 m/min on an 15% grade during the testing period. The day before and the day of an experiment rats were not trained.

Radiochemical purity of uniformly labelled ^{14}C isotopes (ICN Irvine, CA) was certified to exceed 98%, which we confirmed by two dimensional paper chromatography (9). The specific activities of stock isotopes obtained were: L-alanine, 120 mCi/mM; D-glucose, 184 mCi/mM; and L-Leucine, 190 mCi/mM. The stock isotopes were adjusted so that a 250 µl injection dose included 5 µCi isotope, 50 µl of 154 mM sodium phosphate buffer (pH 7.4), and 154

NaCl. Dosages were administered intraperitoneally (ip).

Following an overnight fast, experimental animals to be studied received 10 min of preliminary rest or exercise in the metabolic chamber. After administration of the isotope, each rat was returned to the metabolic chamber and continued to either rest or exercise for 2 h. Oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were determined continuously for 2 h following isotope injection by the rapid flow, open circuit indirect calorimetry system described by White and Brooks (48). In this system ambient air was drawn at the rate of 5 L/min around the bottom edges of the chamber past the animal, and into a mixing manifold. One tenth of the "expired" flow was then dried by passing through anhydrous $CaSO_4$, and then directed through Beckman LB2 CO_2, and Applied Electrochemistry S3A O_2 analyzers. Outputs of these analyzers were recorded on a Brush 440 recorder, with 0.5% full scale deflection. After compositional analysis the gas was directed to one of twelve glass CO_2 trapping columns which were arranged in series. Each trap contained 50 ml ethanolamine-methylcellusolve (EMC), (1:2, v:v). Following isotope injection, CO_2 was trapped continuously for six 5-min periods, and nine 10-min periods. Three traps had to be used twice during the course of each 2 h experiment. Cleansing of traps was accomplished with two 50 ml washes of 95% methanol, following by forced air drying. Duplicate 5 ml aliquots of EMC from each trap were mixed with 15 ml of Aquasol II liquid scintillation cocktail (New England Nuclear, Boston, MA), and β-emission was quantified on a Packard Tricarb 3385 liquid scintillation counter. Counting efficiency was assessed using both internal and external standards and ranged from 60 to 65%.

$\dot{V}O_2$ and $\dot{V}CO_2$ were determined from the recorder tracings of respiratory gases leaving the metabolic chamber. Intervals corresponding to the $^{14}CO_2$ trapping periods were identified, and ten F_EO_2 and F_ECO_2 values were taken and used to calculate mean values for that time interval. Knowing

the metabolic chamber flow and the percents of O_2 extracted and CO_2 produced across the chamber, $\dot{V}O_2$ and $\dot{V}CO_2$ were calculated as described previously (48). Specific activity of expired CO_2 ($SACO_2$) was calculated from CO_2 trap activity and corresponding $\dot{V}CO_2$, assuming 22.26 ml CO_2/mMole. $\dot{V}O_2$, $\dot{V}CO_2$, R, external work rate, percent ^{14}C recovered as $^{14}CO_2$ and $SACO_2$ were calculated.

One week following completion of all metabolite infusions, 18 trained rats were used to study CO_2 retention characteristics. At each metabolic rate, six animals were injected with 5 µCi of [U-^{14}C]-$NaHCO_3$ in 250 µl of 154 mM bicarbonate buffer, pH 8.

In a parallel study, 28 rats were trained and otherwise treated as described above. At one of seven time intervals (2.5, 5, 15, 30, 60, 90, and 120 min) in each metabolic condition, 2 ml blood was sampled by cardiac puncture from a rat. Enzymatic assays (Sigma, Inc. St. Louis, MO) for glucose and lactate concentrations were performed on neutralized perchloric acid blood extracts. Blood was withdrawn on three occasions from each rat, at least one week apart, once at each metabolic rate.

Curves describing the evolution of $^{14}CO_2$ (i.e., DPM of ^{14}C appearing as $^{14}CO_2$) were plotted from the raw data. Also plotted were evolution of $^{14}CO_2$ curves normalized for metabolic rate (i.e., $SACO_2$). Since the CO_2 trapping intervals varied (i.e., six 5-min and nine 10-min intervals) plotted data were normalized to a per minute value. Following isotope injection (i.e., time zero), evolution of $^{14}CO_2$ increased to a maximum at some time post-injection, and then decreased in an exponential fashion with one or two components. Group mean data following the attainment of peak activity for both DPM as CO_2 and $SACO_2$ were plotted on semilog graph paper. From these plots, the $^{14}CO_2$ collection time intervals making up the components of the exponential decay

were assessed. Using the group data to identify curve component intervals, linear regressions were performed on the log transformation of data obtained during individual experiments, the slope of those regressions being equivalent to the exponential rate constants. Curve parameters of descriptive importance included time to peak evolution (TPE), magnitude at peak evolution (MPE), and rate constants of exponential decay. Means and standard errors of the means were calculated for purposes of comparison. Analysis of variance (ANOVA) and Scheffé procedures ($p < .05$) were used to test the statistical significance of mean differences. Minor differences in MPE between tabled means and the plotted means (Figures 2-4) existed since animals reached their MPE at different times.

RESULTS

Oxygen consumptions and carbon dioxide productions were markedly influenced by the intensity of exercise (Figure 1). At rest, metabolic rates were elevated immediately following injection which compared to values later in the 2 h protocol. We attribute this to handling of the animals associated with isotope injection and subsequent activities of animals such as grooming and exploration of the metabolic chamber. A similar but less pronounced effect was observed during running at both 14.3 m/min, 1% grade (easy exercise) and 28.7 m/min, 15% grade (heavy exercise). In addition to the effects of handling, the increased metabolic rate at exercise onset coincided with erratic pacing of animals. When expressed in relative units, several of the $\dot{V}O_2$ values during heavy exercise exceeded 80 $ml \cdot kg^{-1} \cdot min^{-1}$. Respiratory exchange ratios ($R = \dot{V}CO_2/\dot{V}O_2$) are presented in the lower portion of Figure 1. At rest, excitement associated with handling, infusion, and placement in the metabolic chamber increased R as compared to the latter periods of quiescence. At the onset of running at 28.7 m/min and 15% grade, R exceeded unity, but then

declined in an exponentially over time to approximately 0.95 at the end of 2 h. The values of R obtained during running at 14.3 m/min and 1% grade are equal to or slightly less in most instances than those observed in heavy exercise, but the curve configurations were similar. The interaction model ANOVA performed on $\dot{V}O_2$, $\dot{V}CO_2$ and R indicated that the metabolic responses of the rats to rest or two intensities of running did not differ between the substrate injection groups.

Compared to rest, the two exercise tasks resulted in significant alterations in blood lact and glc concentrations (Table 1). Blood lact concentration was directly related to MR.

Evolution of $^{14}CO_2$ following injection of [U-^{14}C]-glc was relatively slow in resting animals (Figure 2A). Peak evolution occurred earlier when running at either intensity as compared to rest, but there was no difference in TPE between the two exercise intensities. With increments in metabolic rate, the magnitude of peak DPM increased following glc injection (Table 2). Specific activities of CO_2 evolved (Figure 2B) demonstrated a similar effect of exercise on the timing of peak evolutions, but the differences in peak magnitudes were not statistically significant (Table 2). As indicated by the magnitude of the negative slope following attainment of peak evolution (Figures 2A and 2B), and the corresponding first component exponential rate constants (Table 2), a direct relationship between metabolic rate and the oxidation of glc was demonstrated. At the end of 2 h, 42.4 $\pm$ 2.4, 67.4 $\pm$ 2.8 and 82.0 $\pm$ 3.4% ($\bar{X} \pm S\bar{x}$) of the ^{14}C injected as ^{14}C-glc were recovered as $^{14}CO_2$ during rest, mild and heavy exercises, respectively (Figure 2C).

Figure 3A depicts the evolution of $^{14}CO_2$ following [U-$^{14}CO_2$]-ala injection at the three metabolic conditions. As seen in Table 2, metabolic rate has no effect on time to peak $^{14}CO_2$ evolution. Magnitude of peak

$^{14}CO_2$ evolution was greater during both exercise intensities than during rest, but there was no difference between MPE at the two exercise intensities. When $^{14}CO_2$ evolution following ala injection was expressed as specific activity (Figure 3B), the attainment of peak evolution was faster at both exercise intensities than during rest. No differences were observed in magnitude of peak $SACO_2$ evolution. Percent recovery of ^{14}C-ala recovered as $^{14}CO_2$ at 2 h were 54.5 $\pm$ 1.8, 70.6 $\pm$ 2.1, and 74.1 $\pm$ 2.7 at the three metabolic rates, respectively (Figure 3C). Figures 3B and 3C indicate a marked similarity between the curves corresponding to two intensities of exercise. Moreover, no significant differences were observed in the rate constants of the first component of exponential decay expressed as either DPM as $^{14}CO_2$ or $SACO_2$, between exercise intensities. The capacity of the rat to oxidize ala appears to be saturated at the mild exercise level.

Figures 4A and 4B reveal the evolution of $^{14}CO_2$ following [U-^{14}C]-leu injection to be the most diminutive of the three substrates studied. The time to peak $^{14}CO_2$ evolution was shortest during heavy exercise than during either rest or mild exercise, which were not significantly different from each other (Table 2). With leu the magnitude of peak DPM evolution was directly related to metabolic rate. The only significant difference in magnitude of peak $SACO_2$ evolution occurred between rest and heavy exercise. Percent recoveries of ^{14}C-leu as $^{14}CO_2$ were 18.3 $\pm$ 1.5, 35.5 $\pm$ 5.4 and 44.7 $\pm$ 3.3 at the three metabolic rates, respectively (Figure 4C). The rate constants (Table 2) of the first component exponential decay curves following leu injection (Figures 4A and 4B) demonstrate an enhanced rate of oxidation when comparing data at the heavy exercise level to data obtained during either rest or mild exercise.

The evolution of $^{14}CO_2$ following injection of 5μCi-[U-^{14}C]-$NaHCO_3$ reached its peak in the first or second trapping interval, irrespective of metabolic rate and then decayed in a three component exponential fashion. The rate constants of the first component of DPM as $^{14}CO_2$ were -.0851, -.0893, and -.1032 at the three increasing metabolic rates. When expressed as $SACO_2$, the three rate constants were -.0696, -.0811, and -.0914. These rates of $^{14}CO_2$ washout are 3 to 10 times greater than those observed with the three other metabolites.

DISCUSSION

These results on metabolic responses of rats to exercise reveal some quantitative differences in comparison with data obtained on humans. It is generally recognized that the resting metabolic rate in small mammals is relatively elevated in comparison to that in larger species (45). Consequently, despite the fact that $\dot{V}O_2$ max exceeds 80 $ml \cdot kg^{-1} \cdot min^{-1}$ in rats (48), high resting $\dot{V}O_2$ limits the capacity for respiratory expansion during exercise. Since the rat appears to be capable of only 3 to 5 METS, the elevation in blood lactate in response to mild exercise (Table 1) may be explained. Blood lactate concentration correlated .9206 with $\dot{V}O_2$, which supports this interpretation.

The effect of external work rate as imposed by treadmill running had marked effects on $\dot{V}O_2$ and $\dot{V}CO_2$ (Figure 1). The higher values of $\dot{V}O_2$ and $\dot{V}CO_2$ observed initially after injection were associated with spontaneous movements on the part of the animals. This was most apparent at rest where animals would groom and explore the metabolic chamber before curling up in a corner. Rats frequently responded to the easy exercise task (14.3 m/min on a 1% grade with erratic pacing and consequently the O_2 and CO_2 tracings showed greater relative variance than in hard exercise (28.7 m/min on a 15%

grade) where the rats had to continuously work to avoid the shocking prods.

Values of respiratory exchange are consistent with those expected on the basis of the variations in the curve for $\dot{V}CO_2$ and $\dot{V}O_2$ (Figure 1). Across work loads there was a direct relationship between R, $\dot{V}O_2$ and $\dot{V}CO_2$. Correlation coefficients between R and $\dot{V}O_2$ and $\dot{V}CO_2$ were 0.83 and 0.93, respectively. This relationship of R and metabolic rate was most apparent at rest where spontaneous activity after isotope injection was associated with much higher values of R than later on. Initially after injection at rest, values of respiratory exchange were comparable to those in exercise. Higher values of R immediately following injection were associated with elevated blood lactate concentrations. Over the duration of the experimental period, there is a definite trend for R to decline. This may be indicative of an increased contribution by fat catabolism to energy flux. The observed values of R (0.8) in the quiescent rat would be expected in a post-abortive animal. In comparing values of R between the two work loads it was observed that R was slightly higher in heavy than in easy exercise. These results suggest that the relative utilization of carbohydrate is increased during exercise and that the rat responds to even easy exercise by augmentations of glycogenolysis and glycolysis. This interpretation is supported by the data on blood lactate concentration (Table 1)and is consistent with the observation of a limited capacity for respiratory expansion in the rat.

Presented in Figure 5 are data on $^{14}CO_2$ evolution obtained from animals which were treated as described earlier, except that jugular catheters had been implanted (42). Data presented are for $^{14}CO_2$ evolution following either intraveneous (iv.) or intraperitoneal (ip.) injection of [U-^{14}C]-glc or [U-^{14}C]-lact. The radioactivity peaks earlier following iv. injection, but later on recovery of label is similar irrespective of mode of injection. If the peak data point is disregarded, iv. and ip. injections produce identical

$^{14}CO_2$ decay rate constants. Originally it was our intent to inject all animals via a jugular catheter, but it became apparent that following catheterization, even allowing several weeks for retraining and recouperation from surgery, that animals could not again run continuously for 2 hours at 28.7 m/min, 15% grade. Since veterinary autopsy of apparently healthy catheterized animals revealed no pathological indices, we suspected that the jugular-right atrial catheters might have impaired venous return. In view of the close correspondence between $^{14}CO_2$ evolution curves following iv. and ip. injections, the difficulty in maintaining catheters patent in exercising animals for weeks without the use of heparin, and the difference in exercise capacity induced by catheterization, we decided to utilize the ip. mode of injection.

The evolution of $^{14}CO_2$ following injection of [U-^{14}C]-$NaHCO_3$ appeared faster than in any of the substrate injection groups. The rate constants of the first component of exponential decay following attainment of peak evolution were three to ten times greater than those observed in the substrate injection groups. We interpret this to mean that evolution of $^{14}CO_2$ following injection of ^{14}C-substrate was indicative of oxidation and not merely bicarbonate washout. Our bicarbonate washout rate constants are consistent with those observed by Morris (39).

Results of the present investigation are consistent with the hypothesis that the metabolism of particular amino acids is integrally linked to the energy transductions contributing to the carbon flow sustaining exercise. The present results are the first to our knowledge which indicate that the oxidations of any amino acids are enhanced during muscular exercise <u>in vivo</u>. Historically, the assumption of insignificant protein and amino acid catabolism in exercise, has led to the use of non-protein RQ tables (49) for estimation of the fractions of carbohydrate and fat utilized during exercise,

and for determination of caloric equivalents of $\dot{V}O_2$. The results reported here indicating enhanced amino acid oxidation in exercise may require reevaluation of these applications of non-protein RQ tables.

Inspection of all aspects of the evolution of $^{14}CO_2$ following injection of [U-^{14}C]-glc (Figures 2A and 2C) indicates an enhanced rate and magnitude of glc utilization proportional to increased metabolic rate. This observation does not hold true following the injection of [U-^{14}C]-ala (Figures 3A-3C) where the magnitude at peak of $^{14}CO_2$ evolution expressed as DPM is greater in exercise than at rest, but no difference exists between the two levels of exercise. We interpret these observations to indicate that the ala pool size does not increase in direct proportion to increments in metabolic rate. The rate of oxidation of ala (Figure 3B, Table 2) is greater during exercise than at rest, but no differences were apparent between the two exercise intensities. This is in contrast to the observation made with glc. It appears that the capacity to expand ala oxidation in exercise is limited. The close correspondence between the $SACO_2$ curves following ala infusion (Figure 3B) and cumulative percent of activity recovered as $^{14}CO_2$ at the two exercise intensities (Figure 3C) suggest that the pathways for ala oxidation are saturated even in easy exercise. This interpretation is further supported in Figure 6, where the relationships between $SACO_2$ first component exponential rate constants for ala and metabolic rates are not linear. In contrast to the case of ala, where oxidation does not increase in direct proportion to metabolic rate, the oxidation of glc is linearly related to MR, and for leu oxidation increases disproportionately in relation to MR (Figure 6).

Comparison of the time to peak and cumulative percent recoveries following ala injection (Figures 3A and 3C) with those following glc injection (Figure 2A and 2C) indicate greater recoveries of label at rest and during

easy exercise for ala than for glc. The evolution of $^{14}CO_2$ following [U-^{14}C]-ala infusion does not, therefore, simply reflect the conversion of ala to glc prior to oxidation. Though tempting, a direct quantitative comparison of $^{14}CO_2$ and $SACO_2$ peak heights between glc and ala is not justified. The seemingly large oxidation of ala in comparison to that of glc following pulse infusion of tracer must be considered in relation to the pool sizes of each metabolite. Since the pool size of glc (Table 1) is undoubtedly much larger than that of ala (1,14,35), comparisons of $^{14}CO_2$ peak heights following pulse tracer injections cannot be directly compared acoss metabolites.

The oxidation of leu (Figures 4A-4C) follows a pattern similar to that observed for glc. Peak heights and quantitative recoveries following leu injection are small in comparison with those of either glc or ala. Since the pool size of leu in plasma and muscle (2,3) are small in comparison to those of the other two metabolites, the magnitude of leu oxidation must be viewed as being relatively small. Examination of the cumulative recoveries of radioactivity and the slopes of the $SACO_2$ curves following leu injection as a function of metabolic conditions does, however, indicate a relative enhancement of leu oxidation during muscular exercise.

In this study we have used the first component exponential rate constant of $^{14}CO_2$ production to reflect the oxidation of the trace. The existence of second or third rate constants for ala and leu probably reflects the conversion of tracers to other metabolites prior to decarboxylation. We do not feel justified to speculate upon these processes at this point.

The present results on $^{14}CO_2$ evolution following pulse infusions of tagged ala and leu in intact animals provide results which complement those of previous investigations by others. Several groups of investigators (10,27,30,36,40,43) have independently reported that leu can be oxidized

by rat skeletal muscle *in vitro*. The present results are consistent with the interpretation that elevated rates of metabolism in skeletal muscle are associated with an increase in the rate of leu oxidation. The present results are also consistent with those of Ahlborg, *et al*. (4) indicating skeletal muscle uptake of BCAA in contracting human skeletal muscle during prolonged exercise. Results of the present study are the first which, to our knowledge, provide direct evidence that BCAA are oxidized as fuels for muscle work.

The work of Ahlborg, *et al*. (4) has previously implicated the "glucose-alanine cycle" in the metabolic adjustments to prolonged exercise in humans. Results of the present study indicate $^{14}CO_2$ production following ^{14}C-ala injection to occur before and to be greater in magnitude than following glc injection in two of the three metabolic conditions studied. The present results, therefore, indicate that ala plays a role other than as a peripheral to central nitrogen and carbon carrier during exercise. Ala also appears to be a readily oxidizable substrate for the performance of muscular work.

The apparent saturation of avenues of ala oxidation in hard exercise, which did not occur with leu, the difference in first order rate constants, and the quantitatively different recoveries of the two tracers leads to the interpretation that the metabolism of each is not exclusively and directly linked as might be predicted on the basis of a "branched-chain amino acid-alanine cycle" model. No doubt the metabolism of these two and other amino acids will be demonstrated to be linked to carbon flow during exercise in ways which at present cannot be predicted. De Rosa and Swick (13) have investigated intracellular distributions and Michaelis constants for mitrochondrial and cytoslic alanine aminotransferase isoenzymes. Their data suggest alternative pathways of alanine metabolism in addition to the glucose-alanine cycle.

Although the present results on intact animals *in vivo* offer no direct evidence of the sites of amino acid utilization in exercise, the data do support the hypothesis advanced. Even if increments in the rates of ala and leu oxidation during exercise are eventually shown to be compensated for by decrements in the basal rate of oxidation of other carbon sources, the present results still require reevaluation of traditional views of amino acid utilization in exercise.

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FOOTNOTES

Abbreviations used:

ala:	alanine
AMP:	adenosine monophosphate
Ci:	curie = 2.22×10^{12} DPM
CoA:	coenzyme A
DPM:	disintegrations per minute
EMC:	ethanolamine methylcellusolve
$F_E CO_2$:	fraction of dry expired gas appearing as CO_2
$F_E O_2$:	fraction of dry expired gas appearing as O_2
glc:	glucose
IMP:	inosine monophosphate
ip.:	intraperitoneal
iv.:	intravenous
lact:	lactic acid
leu:	leucine
METS:	multiples of the resting metabolic rate
MPE:	magnitude of peak $^{14}CO_2$ evolution
MR:	metabolic rate
$SACO_2$:	specific activity of CO_2
TPE:	time to peak of $^{14}CO_2$ evolution

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FIGURE LEGENDS

Figure 1. Metabolic responses of female Wistar rats (n=18) to 2 h of rest and two intensities of treadmill running. Upper data are $\bar{X} \pm S\bar{x}$ where open symbols = $\dot{V}CO_2$ and solid symbols = $\dot{V}O_2$. Respiratory exchange ratios ($R=\dot{V}CO_2/\dot{V}O_2$) are group mean values with geometric symbol shapes corresponding to metabolic rates as indicated above.

Figure 2. (A) Evolution of $^{14}CO_2$ (DPM), (B) $^{14}CO_2$ specific activity, and (C) cumulative percent recovery of ^{14}C as $^{14}CO_2$ following injection of 5 μCi [U-^{14}C]-glucose in resting or running female Wistar rats; n=6 at each metabolic rate. Values are group means.

Figure 3. (A) Evolution of $^{14}CO_2$ (DPM), (B) $^{14}CO_2$ specific activity, and (C) cumulative percent recovery ^{14}C as $^{14}CO_2$ following injection of 5 μCi [U-^{14}C]-alanine in resting or running female Wistar rats; n=6 at each metabolic rate. Values are group means.

Figure 4. (A) Evolution of $^{14}CO_2$ (DPM), (B) $^{14}CO_2$ specific activity, and (C) cumulative percent recovery ^{14}C as $^{14}CO_2$ following injection of 5 μCi [U-^{14}C]-leucine in resting or running female Wistar rats; n=6 at each metabolic rate. Values are group means.

Figure 5. Evolution of $^{14}CO_2$ (DPM) following ip. or iv. injection of 5 μCi [U-^{14}C]-glucose or [U-^{14}C]-lactate. Data collected over 2 h of rest or during 40 min running (28.7 m/min and 1% grade) followed by 80 min post-exercise recovery, n=3, except iv. glc where n=5.

Figure 6. Rate constants of $^{14}CO_2$ S.A. evolution as a function of metabolic rate ($\dot{V}O_2$). The rate constants ($\bar{X} \pm S\bar{x}$) are for the first exponential component of the evolution curve following attainment of peak activity. $\dot{V}O_2$ values (ml/min) $\bar{X} \pm S\bar{x}$ are for the time interval corresponding to the first exponential component.

0 0 0 0 4 7 1 0 2 6 4

Table 1. Average blood glucose and lactate concentraiton ($\bar{X} \pm S\bar{x}$) during 2 h of rest, easy or hard running exercise. Values represent 28 independent determinations.

Metabolic Condition	Glucose (mM)	Lactate (mM)
Rest	3.58 ± 0.16	3.86 ± 0.37
Running at 14.3 m/min on a 1% grade	5.74 ± 0.46	6.68 ± 0.49
Running at 28.7 m/min on a 15% grade	4.33 ± 0.22	8.54 ± 0.34

Table 2. Curve parameters at (A) rest, (B) running at 14.3 m/min on a 1% grade (easy exercise), and (C) running at 28.7 m/min on a 15% grade (hard exercise) for (1) DPM and (2) $SACO_2$ data. Parameters include time to peak evolution (TPE) and magnitude at peak evolution (MPE ↑) following injection of 5 μCi [U-^{14}C]-glucose, -alanine, or -leucine. The slope (a_1), standard error of the slope (Sa_1) and the goodness of fit (r^2) of the linear regression of log transformed data following attainment of peak evolution are also included. The slope is equivalent to the rate constant of exponential decay. CI = time interval (min) on which the exponential component is based. Differences between group means were assessed statistically (Scheffé analyses) for all parameters except CI, Sa, r^2. Values are $\bar{X} \pm S\bar{x}$.

	[U-^{14}C]-METABOLITE INJECTED		
PARAMETER	GLUCOSE	ALANINE	LEUCINE
A. Rest			
1) DPM			
TPE	7.33±0.49 (ALEH)	3.33±0.33	4.00±0.00 (H)
MPE	6.31±0.45 (EH)	10.86±0.71 (EH)	4.88±0.53 (H)
CI	50-120	20-120	20-90
a_1	-.0086±.0020 (LEH)	-.0159±.0006 (LEH)	-.0283±.0020 (H)
Sa_1	.0032±.0007	.0018±.0003	.0033±.0005
r^2	.55±.14	.90±.02	.91±.03
2) $SACO_2$			
TPE	8.33±.0.71 (ALEH)	4.50±0.34 (EH)	4.00±0.00
MPE	21.81±1.60	27.85±1.62 (L)	12.48±1.42 (H)
CI	50-120	20-120	20-90
a_1	-.0093±.0013 (LEH)	-.0131±.0006 (LEH)	-.0211±.0017 (H)
Sa_1	.0023±.0007	.0007±.0003	.0026±.0012
r^2	.77±.07	.96±.02	.88±.09

Table 2. (cont'd)

B. 14.3 m/min 1%:

1) DPM

TPE	5.00±0.68 (A)	3.00±0.26	4.33±0.33 (H)
MPE	12.85±1.19 (H)	18.29±1.03 (L)	8.60±0.82 (H)
CI	25-120	15-80	25-80
a_1	-.0193±.0018 (H)	-.0252±.0014	-.0332±.0054
Sa_1	.0008±.0002	.0016±.0003	.0025±.0004
r^2	.98±.01	.98±.01	.97±.01

2) $SACO_2$

TPE	5.33±0.67 (A)	2.83±0.31	5.00±0.63 (H)
MPE	23.92±2.16	25.75±2.45	15.71±1.25
CI	25-120	15-80	25-80
a_1	-.0190±.0021 (H)	-.0229±.0014	-.0305±.0047 (H)
Sa_1	.0008±.0002	.0014±.0002	.0018±.0005
r^2	.97±.01	.98±.01	.96±.03

C. 28.7 m/min 15%:

1) DPM

TPE	4.50±0.34 (AL)	3.33±0.21	3.00±0.00
MPE	20.89±1.42	17.15±1.54	15.34±1.65
CI	20-120	20-80	15-60
a_1	-.0292±.0018 (L)	-.0260±.0009 (L)	-.0447±.0007
Sa_1	.0013±.0002	.0012±.0002	.0025±.0006
r^2	.98±.01	99±.01	.99±.01

2) $SACO_2$

TPE	4.17±0.31	3.00±0.00	3.17±0.17
MPE	25.59±2.44	21.40±0.88	18.11±1.46
CI	20-120	20-80	15-60
a_1	-.0283±.0021 (L)	-.0261±.0015 (L)	-.0438±.0008
Sa_1	.0014±.0002	.0012±.0002	.0019±.0003
r^2	.97±.01	.99±.01	.99±.003

Table 2 (cont'd.)

[§]CO_2 trapping intervals have been numbered sequentially with the 0-5 min interval assigned 1, and the 110-120 min interval assigned 15.

[†]Values are either DPM x 10^4 or $SACO_2$ x 10^4. Designation of the statistical significance of group differences (p. <.05):

1. At a given metabolic rate, differences between substrate injection groups:

   'A' : glc-ala

   'L' : glc-leu or ala-leu

2. Within a specific substrate injection group, differences between metabolic rates:

   'E' : rest-easy exercise

   'H' : rest-hard exercise or easy-hard exercise.

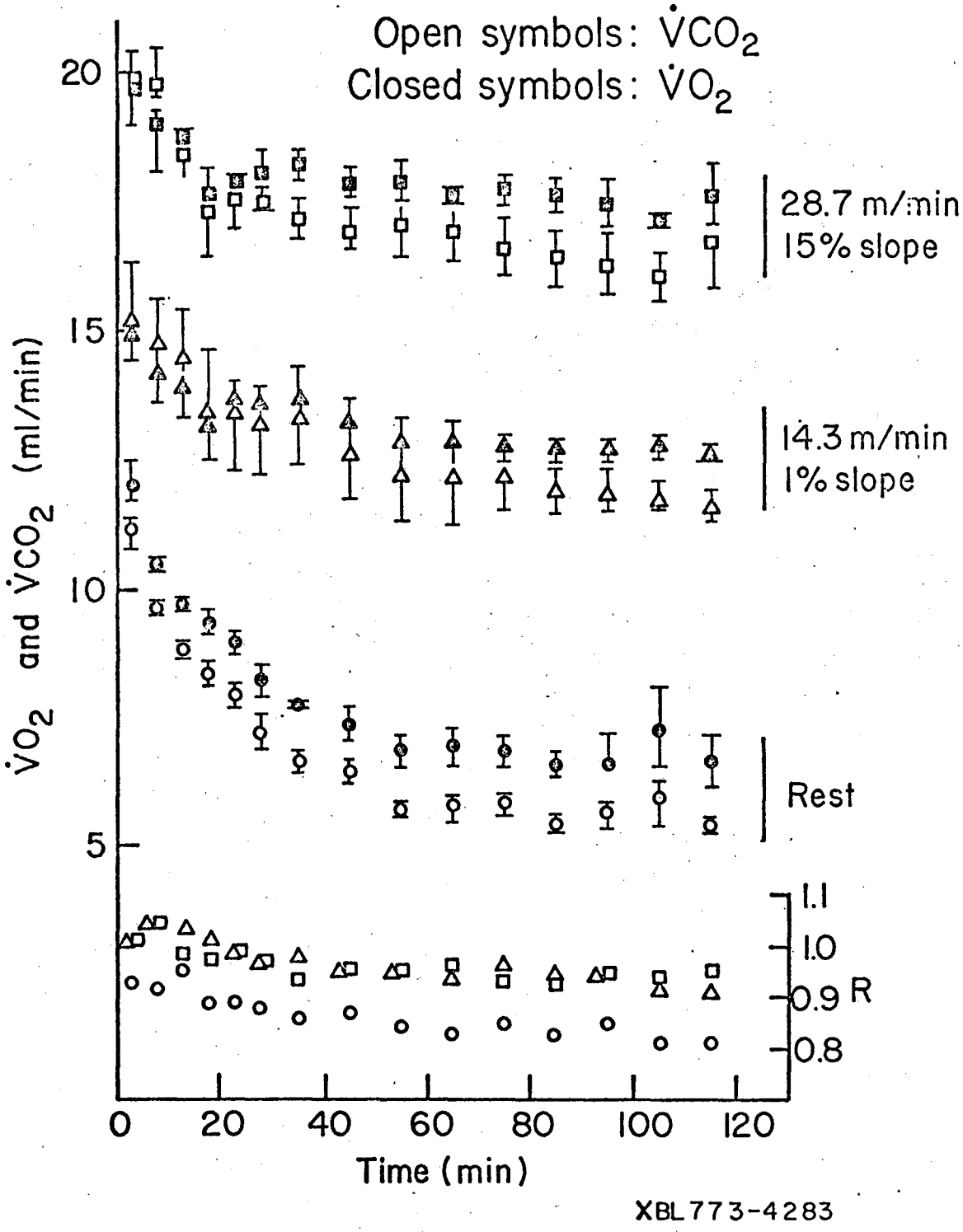

Open symbols: $\dot{V}CO_2$
Closed symbols: $\dot{V}O_2$
28.7 m/min
15% slope
14.3 m/min
1% slope
Rest
$\dot{V}O_2$ and $\dot{V}CO_2$ (ml/min)
20
15
10
5
1.1
1.0
0.9
0.8
R
0
20
40
60
80
100
120
Time (min)
XBL773-4283

Fig. 1

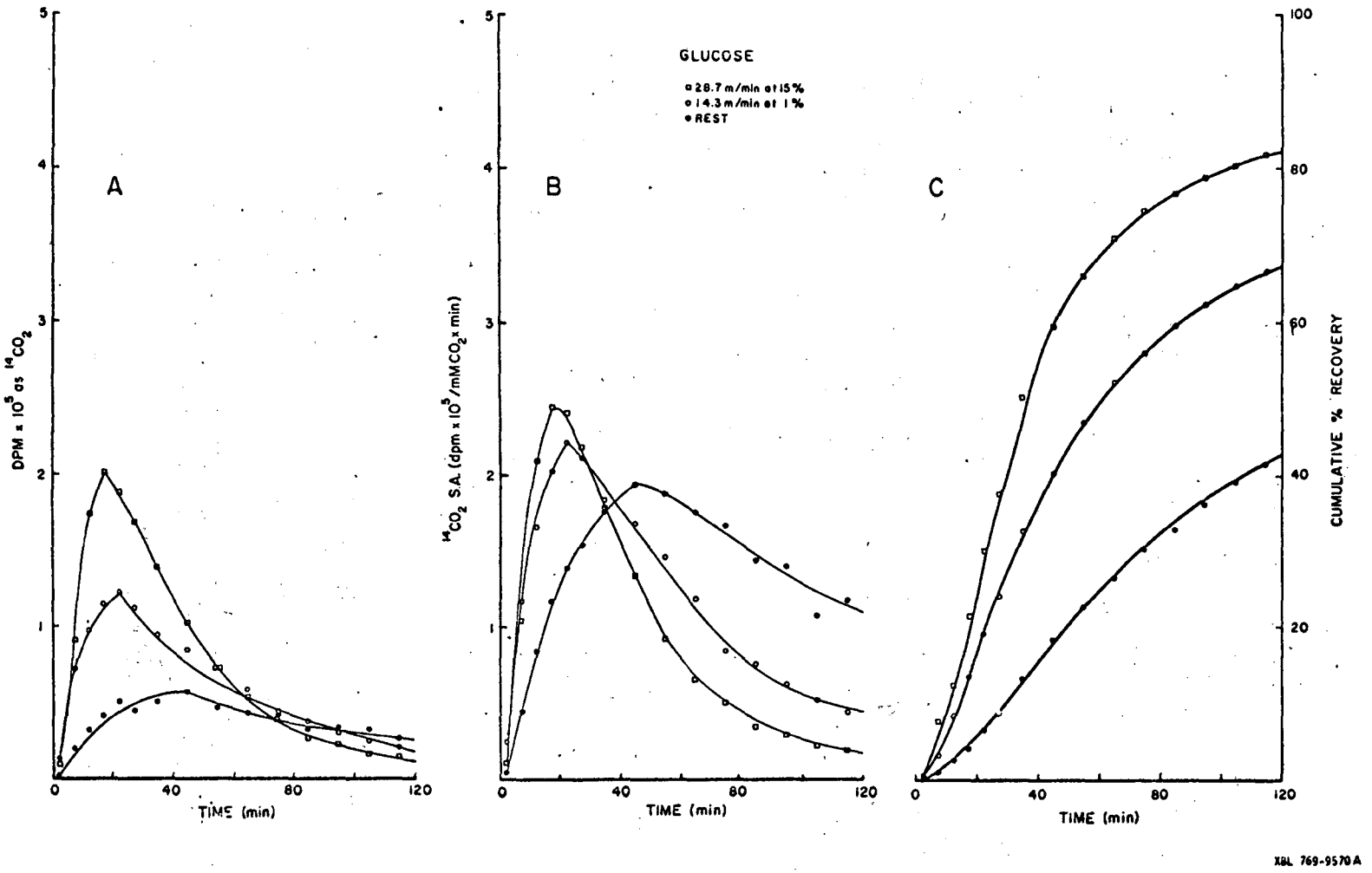

GLUCOSE
28.7 m/min at 15%
14.3 m/min at 1%
REST
A
B
C
DPM x 10^5 as 14CO2
14CO2 S.A. (dpm x 10^5/mMCO2 x min)
CUMULATIVE % RECOVERY
TIME (min)
TIME (min)
TIME (min)
XBL 769-9570A

Fig. 2

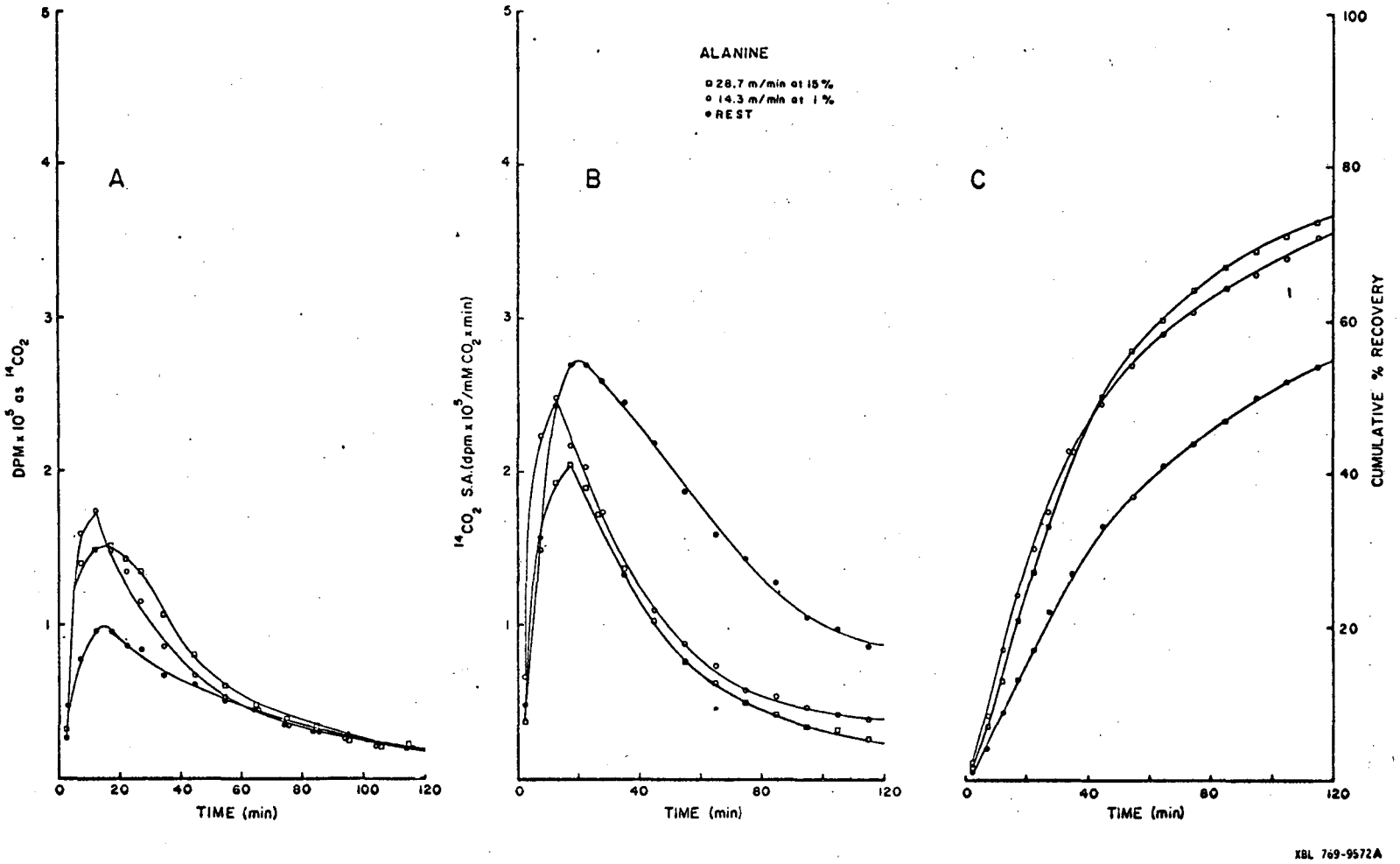


Fig. 3

0 0 4 0 4 7 1 0 2 6 7

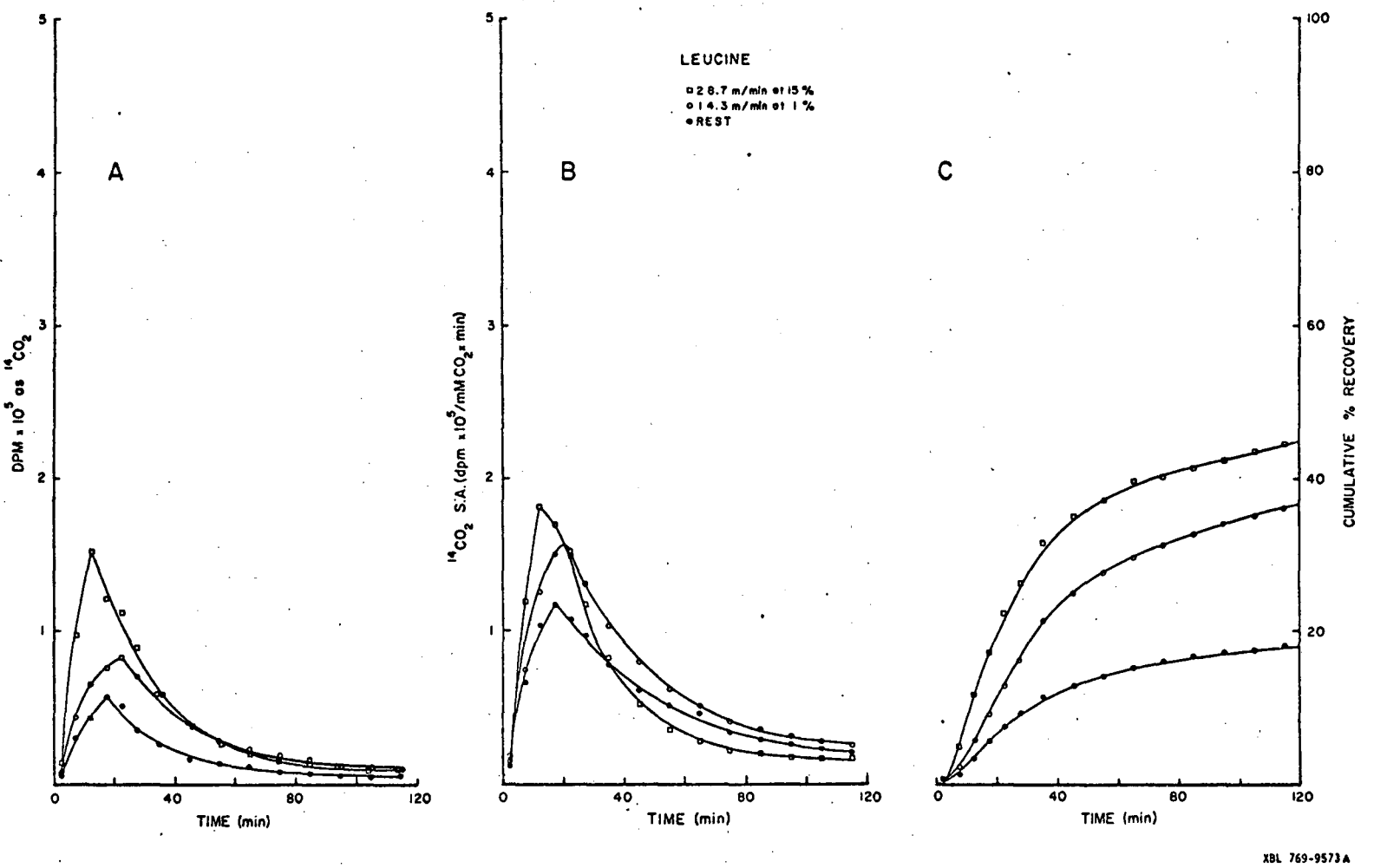
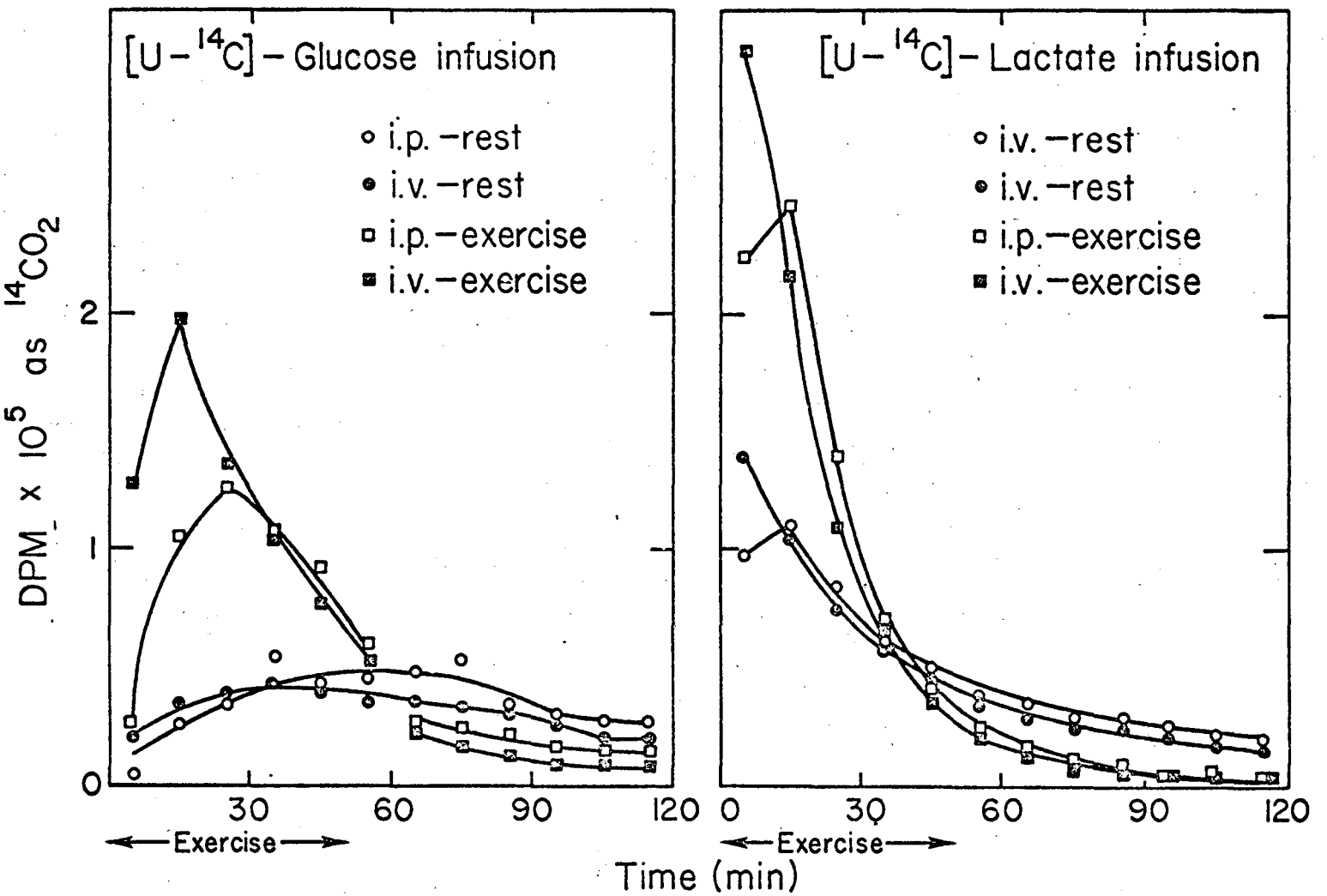

LEUCINE
□ 28.7 m/min at 15 %
○ 14.3 m/min at 1 %
● REST
A
B
C
DPM x 10^5 as 14CO2
14CO2 S.A. (dpm x10^5/mM CO2 x min)
CUMULATIVE % RECOVERY
TIME (min)
TIME (min)
TIME (min)
XBL 769-9573A

Fig. 4



XBL 773-4282

Fig. 5

0 0 0 0 4 7 1 0 2 6 8

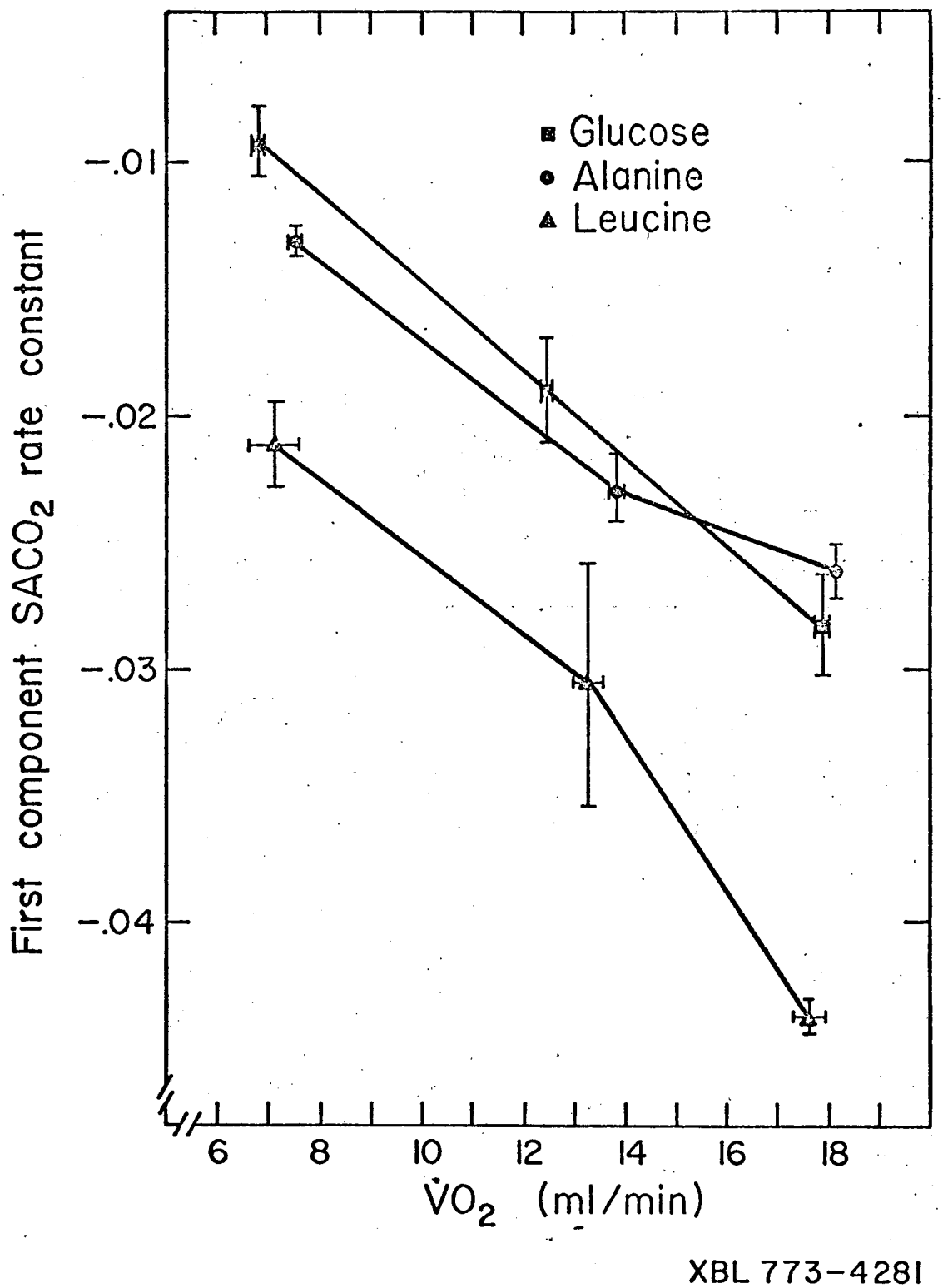

Glucose
Alanine
Leucine
-.01
-.02
-.03
-.04
First component SACO2 rate constant
6
8
10
12
14
16
18
V̇O2 (ml/min)
XBL 773-4281

Fig. 6

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