J. KOREAN SOC. FOOD NUTR.
13(4) 437~443 (1984)

Effects of Exercise on Rat Skeletal Muscle Perfused with Glucose

Sung-Hee Cho and Jae-Man Yoon*

Dept. of Food Science and Nutrition, Hyo Sung Women's Univ., Taegu, Korea
**Dept. of physical Education, Ball State Univ., Indiana, U.S.A.*
(Received September 2, 1984)

포도당으로 Perfusion한 쥐의 다리근육에 運動이 미치는 영향

조 성 희 · 윤 재 만*

효성여자대학교 식품영양학과, *볼스테이트대학 체육교육학과
(1984년 9월 2일 접수)

요 약

포도당으로 perfusion한 쥐의 뒷다리 근육에 전기자극으로 근육운동을 유도하여 대사물의 농도변화를 측정한 결과는 아래와 같다.

근육내의 구연산회로대사물, 피루빅산, 젖산등의 농도가 상당히 증가하였고 알라닌, 글루타믹산, 아스파틱산 등의 아미노산은 약간 증가하였고, 크레아틴 포스페이트, ATP의 농도가 급강하 한데 반하여 AMP와 암모니아의 농도가 증가하였다. 이 결과는 운동시에 에너지대사를 촉진시키는 구연산회로 대사물 일부는 아미노산으로부터 transamination 반응을 통하여 공급되고, 일부는 purine nucleotidecycle 반응을 통하여 아스파틱산으로 공급되고 있음을 밝혔다.

Introduction

In contrast to liver and some other organs, the function of the citrate cycle is in muscle almost, if not exclusively, a catabolic one. It was formerly held that pathways for the net synthesis and consumption of cycle intermediates were virtually absent in muscle[1], but it is now established that pyruvate carboxylase (E. C. 6. 4. 1. 1)[2,3], phosphoenolpyruvate carboxykinase(E. C. 1. 1. 1. 32)[4,5], and NAD^+-and $NADP^+$-linked[6~8] "malic" enzymes(E. C. 1. 1. 1. 39 and 1. 1. 1. 40, respectively) are present in significant amounts in various types of skeletal muscle. It would be expected that muscle is capable of intricate control of the level of citrate cycle intermediates in order to assure optimal aerobic energy production under a variety of conditions, and that the above enzymes play a role in this homeostasis. Indeed, we have recently demonstrated[9] that there is continuous flux of carbon into the citrate cycle pool, and an equal compensating flux from the cycle, thus attaining a constant level of intermediates in a given defined metabolic or physiological condition. The rates of these opposing fluxes may be altered resulting in an altered new steady-state level of cycle intermediates. It may be expected that an elevation in the size of the pool of intermediates would be beneficial in accommodating accelerated respiration during and after muscular contraction. It has been shown that the citrate cycle

pool is acutely elevated by exercise.[10]

Therefore, we have examined the changes in concentrations of citrate cycle intermediates, adenine nucleotides and phosphocreatine, some amino acids as well as glycolytic products in perfused skeletal muscle, the acute contraction of which was induced by electrical stimulation of both fermoral nerves.

Materials and Methods

Rat hindquarters were perfused with 5 mM glucose and 12.5 munit/ml insulin exactly as described by Lee and Davis.[9]

After an initial 50 ml washout, perfusions were conducted either for 15 min or 60 min during which one to three minutes of electrical stimulation were applied.

Isometric muscle contractions were induced by a squarewave electrical impulse generator with Grass S4GR stimulator by attaching an electrode to each of the femoral nerves. The duration of impulse was 5 msec. with a frequency of 5 per sec. The voltage was 0.1~0.2 volt at the beginning. Increases in the voltage was usually necessary to obtain maximal intensity of the contraction throughout the interval of stimulation.

Throughout all perfusions, the flow rate of the medium remained constant at 12~15 ml/min and the mean pressure in the tubing leading to the aortic catheter was 90/70 mmHg. Portions of muscle were freeze-clamped at the appropriate interval, extracted with $HClO_4$ and neutralized[9] and portions of perfusing fluid were also taken for determination of amino acid content.

Citrate cycle intermediates, pyruvate, lactate, ATP, ADP, AMP, and phosphocreatine and amino acids were measured by standard enzymatic assays.[9]

Results and Discusion

1. Effects on Glycolysis

As shown in Table 1, the level of creatine phosphate was reduced to one-fifth of that of resting muscle within one min after muscle started contra-

Table 1. The levels of muscle tissue metabolites in response to electrical stimulation

(μmoles/g wet wt.)

	Control	1 min. Stimulation	3 min. Stimulation
CrP	22.54±1.05(8)	4.36±0.59(6)***	4.04±0.79(6)***
ATP	7.36±0.24(9)	5.43±0.30(8)***	4.43±0.27(8)***
ADP	1.19±0.08(9)	1.22±0.06(8)	1.27±0.07(8)
AMP	0.13±0.01(7)	0.17±0.01(7)**	0.22±0.02(7)***
Cit	0.21±0.01(6)	0.26±0.02(6)	0.14±0.01(6)***
Mal+Fum	0.12±0.02(11)	0.26±0.02(10)***	0.30±0.02(11)***
Cit+Mal+Fum	0.33±0.03	0.52±0.03***	0.44±0.03***
Gln	3.85±0.26(6)	3.66±0.16(8)	4.61±0.33(4)*
NH_3	0.50±0.11(4)	1.76±0.10(4)***	2.44±0.22(4)***
Glu	1.65±0.10(11)	1.36±0.10(11)*	1.12±0.10(11)***
Asp	0.24±0.02(6)	0.32±0.04(6)	0.22±0.03(6)
Ala	1.87±0.08(9)	3.08±0.17(8)***	2.36±0.13(9)***
Glycogen	22.40±1.45(4)	7.55±0.93(4)***	6.15±1.12(4)***
Lactate	1.22±0.13(4)	30.35±1.19(4)***	33.33±1.66(4)***
Pyruvate	0.14±0.01(4)	0.85±0.02(4)***	0.48±0.01(4)***

*** Significantly different from control group, $p<0.005$

** $p<0.05$

* $p<0.1$

Numbers in parentheses are numbers of perfusions. The values are expressed as the means±standard error of the mean(S.E.M.).

ction, indicating that the intensity of exercise employed in this study was close to maximum. It is apparent that during this short-term, intense exercise, muscle glycogen served a major energy source since the level of muscle glycogen dropped sharply within one min of exercise, whereas glucose uptake from extracellular fluid was not increased (Fig. 1). Continuation of exercise from 1 to 3 min did not cause further decrease in muscle glycogen. At the same time, a great amount of lactate accumulated in exercised muscle(32 μmoles/gm tissue after 3 min exercise), and also some was released into the perfusate(about 2 μmoles/gm tissue after 3 min exercise, extrapolated from Fig. 2). Most of the lactate formed during exercise may be looked upon as originating from muscle glycogen which was lost(about 16 μmoles/gm tissue). This indicates that anaerobic glycolysis was greatly stimulated, probably at the step of phosphofructokinase by low ATP/ADP ratio(Table 2), although aerobic glycolysis occurred in parallel to some degree.

The concentration of pyruvate was mostly elevated at one min of exercise, decreasing again somewhat as exercise was prolonged to 3min(Table 1).

It is generally recognized that in man, blood glucose is taken up to some extent by muscle during physical exercise. In a recent study with rat hindquarters[11], exercise induced by sciatic nerve stimulation was reported to have enhanced glucose uptake about ten-fold in both fed and starved rats. However, net glucose uptake(Fig. 1) by exercise was not increased in the present study. This discrepancy is obviously due to the different experimental conditions(3 min of stimulation in this study and 15min of stimulation in the study of Berger *et al.*[11]). It has been observed during heavy forearm exercise(135) that at the onset of exercise there was first a reduction and a reversal of the A-V difference in the concentration of glucose across the forearm, followed by a gradual increase during the next few minutes. This phenomenon of early stage of heavy exercise can be explained as follows. When glycogenolysis occurs, free glucose is formed corresponding to 8~10% of glycogen in

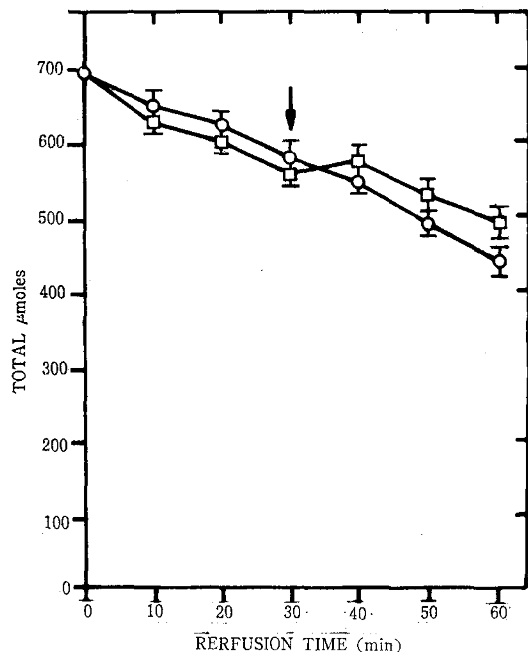


Fig. 1. The effect of muscular exercise on net glucose uptake.
All perfusions were conducted for one hour. In the test perfusions, the femoral nerves were electrically stimulated for a 3min period after 30 min pre-perfusion. Arrow head indicates start of stimulation. Samples from control(circle) and stimulated(triangle) hindquarters were taken from the lower reservoir. Each value represents the mean± S. E. M. for 4 to 6 perfusions.

the debranching process[13].

During conditions of low to moderate rates of glycogenolysis, this glucose is probably rapidly phosphorylated by the hexokinase reaction. But with heavy exercise and rapid glycogen breakdown, intracellular glucose is accumulated when the hexokinase reaction may be operating slowly, due to inhibition mainly by increased glucose-6-phosphate. Under these circumstances, it is likely that glucose transport across the cell membrane and into the cell is slowed down.

During 3 min. exercise, about 48 μmoles of free glucose are expected to have been formed in 30gm muscle(refer to Table 1) by debranching process of

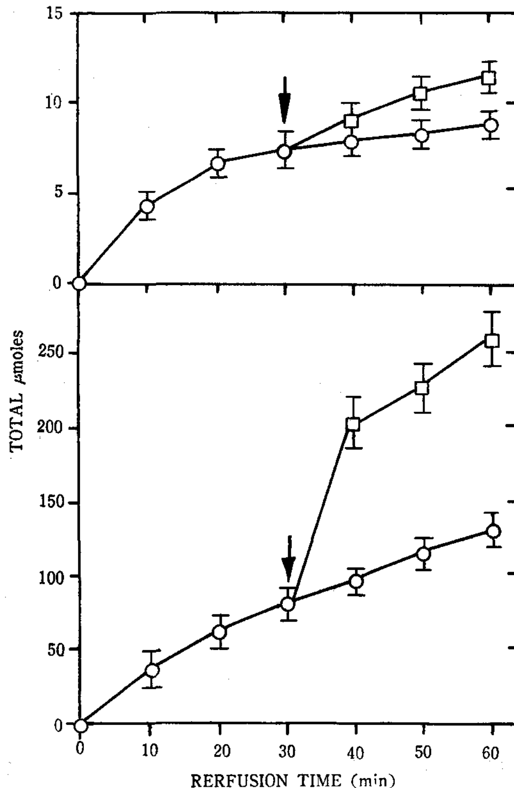


Fig. 2. The effect of muscular exercise on the release of A, pyruvate and B, lactate into perfusate.

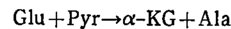
Refer to the conditions described in Fig. 1. Arrow head indicates start of 3 min stmulation.

glycogenolysis in this study. Since there was no change in net glucose uptake during 10 min after 3 min electrical stimulation(Fig. 1), the above amount of free glucose formed must have been consumed, the rate of glucose consumption being about 5 μmoles/min which is almost the same as the rate of glucose uptake in resting muscle.

During recovery, glucose uptake was almost the same as in resting muscle(Fig. 1). There was a rapid increase in the release of lactate from exercising muscle and the rate of lactate release was higher in exercised muscle than in resting muscle during recovery(Fig. 2). On the other hand, compared to the remarkable increase in lactate release, pyruvate release was increased only to a small degree.

2. Effects of Exercise on the Levels of Citric Acid Cycle Intermediates, Alanine, Glutamate and Aspartate

Table 1 shows that muscle contraction effected increases in the levels of citrate and malate plus fumarate. These changes were maximal within 1min of stimulation. Muscle contraction which was continued for 3 min effected small further changes in the level of malate plus fumarate, whereas the citrate content dropped below the resting level. This low citrate value could possibly be due to lower ATP/ADP ratio (Table 2). Tissue pyruvate and alanine concentrations were substantially elevated during 1min stimulation, whereas the concentration of glutamate was decreased. These changes suggest that the rapid elevation in the level of cycle intermediates can be explained at least partly by synthesis of intermediates at the expense of glutamate by a shift in alanine aminotransferase reaction.



$$\text{Glu}+\text{Pyr}\rightarrow\alpha\text{-KG}+\text{Ala}$$

Continued stimulation of muscle from 1 to 3 min decreased both glutamate and alanine. This further decrease in tissue glutamate clearly can not be explained by further shift in the above reaction, since a drop in both pyruvate and alanine in the tissue was observed in the interval between 1 and 3 min of stimulation. Furthermore, the rate of alanine release was not changed by muscle contraction for 3 min(Fig. 3). In a recent study of Felig *et al.*[14], it was reported that in exercising man, alanine is the principal amino acid released by skeletal muscle and that its release is increased

Table 2. Sums and ratios of metabolites in exercising muscle

	Control	1min Stimulation	3min Stimulation
Lac/Pyr	8.7	35.7	69.4
ATP/ADP	6.18	4.45	3.39
$ATP \cdot AMP/ADP^2$	0.68	0.62	0.60
	μ moles/g wet. wt.		
ATP+ADP+AMP	6.82	5.92	5.92
Cit+Mal+Fum	0.33	0.52	0.44
Ala+Asp+Glu	3.68	4.76	3.70
NH_3+Gln	4.34	5.42	7.05

Refer to the conditions described in Table 1.

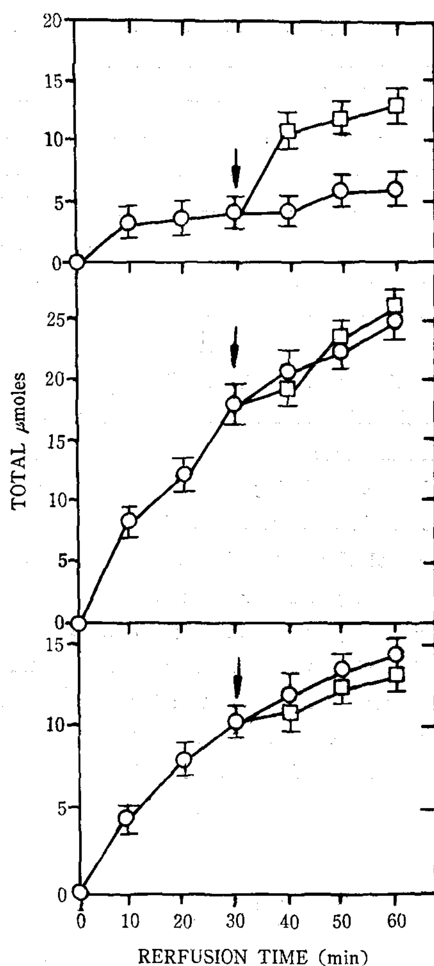
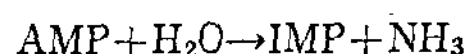


Fig. 3. The effect of muscular exercise on release of A, ammonia; B, glutamine and C, alanine into perfusate.
Refer to the conditions described in Fig. 1. Arrow head indicates start of 3 min stimulation.

by exercise. This increase was not observed in our experiments with the exercising hindquarter. One possible explanation for this discrepancy is that only the muscles innervated by the femoral nerves were actually contracting, whereas the medium was perfusing the entire musculature of the hindquarter. This would result in a dilution of the effect on alanine release from exercising muscles. The release of glutamate was very low in control as well as working muscle, so that it could not contribute significantly to total change in glutamate level. Transamination of glutamate with oxaloacetate, with production of aspartate, does not explain the continued glutamate decrease, since tissue aspartate decreased rather than increased during the 1 to 3 min interval of stimulation. Thus, other metabolic reactions are probably responsible for the observed changes in the level of amino acids and in citric acid cycle intermediates in muscle which was stimulated for 3min.

3. Effects of Exercise on the Concentrations of Adenine Nucleotides, Ammonia and Glutamine

Strenuous exercise of hindlegs for 1min caused a significant decrease in ATP and a further drop with 3 min exercise(Table 1). The concentration of ADP did not change appreciably, but the level of AMP rose progressively during 3 min of stimulation. There was a decrease in the total adenine nucleotides in the 3 min stimulated muscle of about 2.8 μmoles/gm tissue(Table 2). Along with these, there were rapid and marked increases of ammonia in both tissue(Table 1) and perfusate(Fig. 3). Tissue glutamine was also increased whereas release of glutamine into perfusate was not much affected(Fig. 3). It seems probable that loss of total adenine mucleotides is due to conversion of AMP to IMP with release of ammonia, catalyzed by adenylate deaminase which is rather active in skeletal muscle.[15)]



$$AMP + H_2O \rightarrow IMP + NH_3$$

Adenylate deaminase has a relatively high Km for AMP[16)], so that the increase in the concentration of AMP in working muscle probably leads to higher rate of formation of IMP and ammonia. IMP formed in the above reaction is believed to combine with aspartate to give adenylosuccinate, which would be converted into fumarate and AMP to complete the purine nucleotide cycle, with net result of deamination of aspartate to fumarate and ammonia. However, there was no change in the level of aspartate after 3 min exercise compared to that of resting muscle, although there was a transient increase in 1 min stimulated muscle. Increase in aspartate after 1min stimulation is probably due to a rapid equilib-

rium with the increased level of oxaloacetate following the elevated level of malate plus fumarate. As the exercise was contimued to 3 min. The level of aspartate was decreased again to that of control, whereas the sum of malate and fumarate increased slightly more. This appears to indicate that the aspartate which disappeared during 1 and 3 min interval was converted to fumarate and ammonia *via* purine nucleotide cycle. However, the change in the level of aspartate was too small to account for the net formation of ammonia and glutamine during this period. Furthermore, there was virtually no change in the sum of aspartate, glutamate and alanine after 3 min exercise, although a transient increase in the sum was observed after 1min exercise. Therefore, collected data strongly indicates that the major source of nitrogen for the net formation of ammonia and glutamine (2.7 μmoles/gm tissue(Table 2) plus about 0.3 μmole of ammonia released into perfusate(Fig. 3) within 10 min from 1 gm tissue) is adenine nucleotide pool and that glutamate lost during 1 and 3 min interval was used for the synthesis of glutamine. It was observed in the separate work by our collaborator(personal communication) that the level of IMP was remarkably elebated in the exercised muscle(from 0.15 to 3.18 μmoles/gm wet wt.) and was not decreased even after 30 min recovery, suggesting that the purine nucleotide cycle was not functioning rapidly enough for AMP to be recycled.

The results obtained during the intense exercise of short period in this study are taken to imply that citric acid cycle intermediates synthesized during exercise were supplied by amino acids, mostly glutamate *via* alanine aminotransferase, whereas the contribution by purine nucleotide cycle to the synthesis of the cycle intermediates during the exercise and recovery periods was almost negligible.

Summary

Muscular exercise induced by electrical stimulation of femoral nerves in perfused rat hindquarters (5 contractions per sec) in the presence of insulin and glucose effected a rapid increase(*c. a.* two-fold) in the level of citric acid cycle intermediates. The highest values were found within one minute of stimulation. The tissue concent ratios of lactate, pyruvate and alanine increased rapidly on initiation of exercese. Release of lactate also increased rapidly, whereas that of pyruvate was only moderately elevated. In the course of three minute exercese, the sum of alanine, glutamate and aspartate was only transiently elevated. A fall in creatine-p and ATP in the stimulated muscle was accompanied by increases in tissue level of AMP and release of ammonia into perfusing medium. However, the changes in glutamine were small. It is concluded that the pool of citric acid cycle intermediates is expanded during muscular work due (a) to an elevated level of pyruvate, leading to shifts in the levels of alanine and cycle intermediates *via* transamination reactions and (b) to stimulation of the purine nucleotide cycle due to elevated AMP, resulting in generation of cycle intermediates and ammonia at the expense of aspartate.

References

1. Krebs, H. A. : *Proc. R. Soc. Lond. B. Biol. Sci.*, **159**, 545(1964)
2. Ballard, F. J., Hanson, R. W., and Reshef, L. : *Biochem. J.*, **119**, 735(1970)
3. Spydevold, O., Davis, E. J., and Bremer, J. : *Eur. J. Biochem.*, **71**, 155(1976)
4. Opie, L. H., and Newsholme E. A. : *Biochem. J.*, **103**, 391(1967)
5. Nolte, J., Brdiczka, D., and , Pette, D. : *Biochim. Biophys. Acta*, **284**, 497(1972)
6. Davis, E. J., Lin, R. C., and Chao, D. : in *"Energy Metabolism and the Regulation of Metabolic Processes in Mitochondria"*, Mehlman, M. A., and Hanson, R. WL, eds., Academic Press, New York, 211(1972)
7. Lin, R. C., and Davis, E. J. : *J. Biol. Chem.*, **249**, 3867(1974)
8. Frenkel, R. : *J. Biol. Chem.*, **246**, 3069(1971)

9. Lee, S.-H., and Davis, E. J.: *J. Biol. Chem.*, **254**, 420(1979)
10. Goodman, M. N., and Lowenstein, J. M.: *J. Biol. Chem.*, **252**, 5054(1977)
11. Berger, M., Hagg, S. A., Goodman, M. N., and Ruderman, N. B.: *Biochem. J.*, **158**, 191 (1976)
12. Wahren, J., Ahlborg, G., Felig, P., and Jorfeldt, L.: in *"Muscle Metabolism During Exercise"*, Pernow, B., and Saltin, B., eds., Pleuum Press, New York and London, 189 (1971)
13. Field, R. A.: in *"The Metabolic Basis of Inherited Disease"*, McGraw-Hill, New York, 156 (1960)
14. Ahlborg, G., Felig, P., Hagenfeldt, L., Hendler, R., and Wahren, J.: *J. Clin. Invest.*, **53**, 1080(1974)
15. Lowenstein, J. D., and Chappell, J. B.: *FEBS Letters*, **52**, 1-7(1975)
16. Currie, R. D., and Webster, H. L.: *Biochim. Biophys. Acta*, **64**, 30-40(1962)