EFFECT OF SHORT-TERM EXERCISE ON THE CONTENT OF GLUCONEOGENIC METABOLITES IN RAT LIVER AND KIDNEY

R. MUÑOZ-CLARES, J. P. GARCÍA-RUIZ, A. VARGAS and F. SÁNCHEZ-MEDINA
Departamento de Bioquímica, Facultad de Farmacia, Granada, Spain

Received 18 January 1979

1. Introduction

We have reported that muscular exercise (swimming in water at 22°C for 2 h) enhances the activity of phosphoenolpyruvate carboxykinase and the gluconeogenic capacity of rat kidney cortex [1,2]. These effects are probably mediated by the metabolic acidosis which takes place during exercise as result of muscular overproduction of lactate [3].

The increase of renal gluconeogenesis in experimental metabolic acidosis seems to be related to the enhancement of the reaction catalyzed by phosphoenolpyruvate carboxykinase, as indicated by the concentrations of intermediate metabolites of this process [4,5]. Consequently, we have measured the content of gluconeogenic intermediates in kidney after 2 h of swimming in order to obtain further evidence on the relation between the effect of exercise on gluconeogenesis and metabolic acidosis.

2. Materials and methods

Female Wistar rats (150–200 g) were used. The animals were forced to swim in a water bath (22°C) for 2 h. At the end of exercise, they were sacrificed by cervical dislocation. A portion of liver or one of the kidneys was rapidly excised and clamped between metal tongs pre-cooled in liquid nitrogen [6]. The time elapsing between dislocation of the neck and freezing the organ was 8–10 s. The frozen tissue was pulverized in a mortar, extracted with perchloric acid solution and neutralized with KOH, as in [7].

Lactate was determined as in [8]; aspartate as in [9]; malate, as in [10]; pyruvate, phosphoenolpyruvate, 2-phosphoglycerate and 3-phosphoglycerate as in [11]; dihydroxyacetone phosphate, glyceraldehyde 3-phosphate and fructose 1,6-bisphosphate, as in [12]; glucose 6-phosphate and fructose 6-phosphate as in [13].

3. Results and discussion

In the liver from exercised rats the content of lactate was decreased whereas the concentrations of malate, aspartate, 2-phosphoglycerate, fructose 6-phosphate and glucose 6-phosphate were increased (table 1). Assuming that the content of malate and aspartate reflect that of oxalacetate (which is unstable and present in small concentrations in tissues), the rise in malate and aspartate in connection with the absence of a significant enhancement for phosphoenolpyruvate indicate a low activity of phosphoenolpyruvate carboxykinase. Therefore, the decrease in lactate content is not likely due to gluconeogenesis but to oxidative consumption in the tricarboxylic acid cycle. On the other hand, the rise in fructose 6-phosphate and glucose 6-phosphate can be explained by the operation of glycogenolysis. It is well established that glycogen degradation takes place during exercise [14].

The effect of exercise on the renal content of gluconeogenic intermediates is shown in table 2. Like in liver, lactate was decreased and there was a rise in the content of fructose 6-phosphate and glucose 6-phosphate. On the contrary, malate and 2-phosphoglycerate did not charge, asparate and fructose bis-

 Elsevier/North-Holland Biomedical Press

Table 1
Effect of exercise on the hepatic content of intermediates of gluconeogenesis

Experimental conditions	Concentrations of metabolites (nmol/g fresh liver)					
	Lactate	Pyruvate	Malate	Aspartate	Phosphoenol-pyruvate	2-Phospho-glycerate
Control	1470 ± 125 (11)	82 ± 18 (10)	606 ± 46 (9)	695 ± 59 (9)	129 ± 11 (11)	84 ± 11 (12)
Exercise	782 ± 40 (7)[b]	138 ± 23 (7)	1213 ± 122 (7)[b]	2053 ± 202 (5)[b]	141 ± 7 (5)	134 ± 6 (6)[a]
Experimental conditions	3-Phospho-glycerate	Triose-P	Fru-1,6-P_2	Fru-6-P	Glc-6-P	
Control	439 ± 16 (12)	43 ± 8 (9)	21 ± 1 (9)	89 ± 10 (11)	531 ± 52 (11)	
Exercise	455 ± 33 (7)	43 ± 3 (7)	17 ± 3 (7)	152 ± 13 (5)[a]	819 ± 56 (7)[a]	

P values were calculated by Student's *t*-test: [a] $P < 0.01$; [b] $P < 0.001$

Abbreviations: Triose-P, sum of triose phosphate; Fru-1,6-P, fructose 1,6-bisphosphate; Fru-6-P, fructose 6-phosphate; Glc-6-P, glucose 6-phosphate

The rats were forced to swim in a warm water bath (at 22°C) for 2 h. The results are means ± SEM with the number of observations in parentheses

phosphate were decreased and there was a rise in the content of phosphoenolpyruvate.

The decrease in the content of aspartate is noteworthy. Actually, phosphoenolpyruvate carboxykinase is mainly a cytosolic enzyme in rat kidney [15]. Instead, oxalacetate, the substrate for phosphoenolpyruvate formation, is produced in the mitochondria. The transport of oxalacetate from the mitochondria to the cytosol is brought about through its reversible conversion to aspartate when lactate is the gluconeogenic precursor [16] as occurs during exercise. So, the content of oxalacetate under these conditions is reflected by the concentration of aspartate. The rise of phosphoenolpyruvate together

Table 2
Effect of exercise on the renal content of intermediates of gluconeogenesis

Experimental conditions	Concentrations of metabolites (nmol/g fresh kidney)					
	Lactate	Pyruvate	Malate	Aspartate	Phosphoenol-pyruvate	2-Phospho-glycerate
Control	1867 ± 136 (8)	46 ± 2 (8)	392 ± 21 (6)	1122 ± 86 (9)	43 ± 2 (8)	34 ± 8 (8)
Exercise	966 ± 115 (7)[c]	39 ± 4 (5)	377 ± 71 (8)	845 ± 34 (4)	82 ± 10 (5)[b]	35 ± 4 (5)
Experimental conditions	3-Phospho-glycerate	Triose-P	Fru-1,6-P_2	Fru-6-P	Glc-6-P	
Control	152 ± 20 (6)	32 ± 4 (5)	25 ± 2 (6)	16 ± 1 (7)	37 ± 3 (9)	
Exercise	223 ± 34 (5)	43 ± 8 (6)	18 ± 2 (6)[b]	33 ± 4 (5)[b]	118 ± 6 (5)[c]	

P values were calculated by Student's *t*-test: [a] $P < 0.02$; [b] $P < 0.01$; [c] $P < 0.001$

Abbreviations: see table 1

The rats were forced to swim in a warm water bath (at 22°C) for 2 h. The results are means ± SEM with the number of observations in parentheses

with the decrease of aspartate indicate therefore that the activity of phosphoenolpyruvate carboxykinase is enhanced 'in vivo' during exercise, like that reported for acidotic rats [4,5].

Renal gluconeogenesis seems to be also enhanced at the fructose bisphosphatase reaction during exercise as can be deduced from the decrease in the fructose bisphosphate content and the rise of fructose 6-phosphate and glucose 6-phosphate.

In summary, the results described here provide additional support to the hypothesis that the effect of exercise on renal phosphoenolpyruvate carboxykinase is mediated by metabolic acidosis and suggest that renal gluconeogenesis is accelerated 'in vivo' under these conditions.

Acknowledgements

We are very grateful to Professor F. Mayor, Departamento de Bioquímica y Biología Molecular, Universidad Autónoma, Madrid, Spain, for his help and advice. This work was partly supported by a Fellowship from the Ministerio de Educación y Ciencia.

References

[1] Sánchez-Medina, F., Sánchez-Urrutia, L., Medina, J. M. and Mayor, F. (1971) FEBS Lett. 19, 128–130.
[2] Sánchez-Medina, F., Sánchez-Urrutia, L., Medina, J. M. and Mayor, F. (1972) FEBS Lett. 26, 25–26.
[3] Sánchez-Urrutia, L., García-Ruiz, J. P., Sánchez-Medina, F. and Mayor, F. (1975) Biochem. Med. 14, 355–367.
[4] Alleyne, G. A. O. (1968) Nature 217, 847–848.
[5] Hems, D. A. and Brosnan, J. T. (1971) Biochem. J. 123, 391–397.
[6] Wollenberger, A., Ristau, O. and Schoffa, G. (1960) Pflügers Arch. Ges. Physiol. 270, 399–412.
[7] Williamson, D. H., Lund, P. and Krebs, H. A. (1967) Biochem. J. 103, 514–527.
[8] Gutmann, I. and Wahlefeld, A. W. (1974) in: Methods in Enzymatic Analysis (Bergmeyer, H. U. ed) pp. 1464–1468, Academic Press, London, New York.
[9] Bergmeyer, H. U., Bernt, E., Möllering, H. and Pfleiderer, G. (1974) in: Methods in Enzymatic Analysis (Bergmeyer, H. U. ed) pp. 1696–1700, Academic Press, London, New York.
[10] Hohorst, H. J. (1965) in: Methods in Enzymatic Analysis (Bergmeyer, H. U. ed) pp. 328–332, Academic Press, London, New York.
[11] Czok, R. and Eckert, L. (1965) in: Methods in Enzymatic Analysis (Bergmeyer, H. U. ed) pp. 229–233, Academic Press, London, New York.
[12] Bücher, T. and Hohorst, H. J. (1965) in: Methods in Enzymatic Analysis (Bergmeyer, H. U. ed) pp. 246–252, Academic Press, London, New York.
[13] Lang, G. and Michal, G. (1974) in: Methods in Enzymatic Analysis (Bergmeyer, H. U. ed) pp. 1238–1242, Academic Press, London, New York.
[14] Wahren, J., Felig, P., Hagenfeldt, L., Hendler, R. and Ahlborg, G. (1975) in: Metabolic Adaptation to Prolonged Physical Exercise (Howald, H. and Poortmans, J. R. eds) pp. 144–153, Birkhäuser Verlag, Basel.
[15] Flores, H. and Alleyne, G. A. O. (1971) Biochem. J. 123, 35–39.
[16] Williamson, J. R. (1976) in: Gluconeogenesis. Its Regulation in Mammalian Species (Hanson, R. W. and Mehlman, M. A. eds) pp. 165–220, John Wiley and Sons, New York.