The effect of different types of physical exercise on glucose and citrulline synthesis in isolated rat liver parenchymal cells

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Rates of gluconeogenesis from lactate or pyruvate in hepatocytes from untrained rats were not increased by an acute (1 h) bout of exercise (running at 20 m/min). Hepatocytes from rats that had been exercise-trained for 1 month had lower rates of gluconeogenesis from lactate than cells from unexercised controls; the rates with pyruvate were identical. Hepatocytes from livers of trained animals immediately after 1 h of exercise synthesized glucose more rapidly and accumulated more citrulline than cells from resting rats.

Exercise-training *Gluconeogenesis* *Citrulline*

1. INTRODUCTION

During exercise working muscle has an increased energy demand and an increased supply of circulating glucose to muscle is essential. In fed animals glucose is supplied by glycogenolysis [1,2], whereas during fasting animals must rely entirely on gluconeogenesis for maintenance of blood glucose. There is considerable evidence that the process of gluconeogenesis is increased during prolonged exercise [3,4]. It is also known that muscle proteins are involved in exercise metabolism [5–7] and as a consequence of protein catabolism the concentration of some amino acids is increased in liver [8–11], thus enhancing the pool of gluconeogenic substrates. Dohm et al. [12] found that after 6–8 weeks of daily exercise the excretion of urea in rats was increased. In previous research [11] we found that liver mitochondria prepared from acutely exercised untrained rats and trained rats had increased rates of oxidative phosphorylation in state 3, thus providing more ATP for energy-requiring processes. The present study was conducted to investigate alterations in glucose and citrulline synthesis. Previous studies have involved measuring splanchnic uptake of gluconeogenic substrates in man [3] or measuring metabolites of the gluconeogenic pathway in livers of exercised animals [12]. We chose isolated parenchymal cells for our study because they represent the smallest integrated functional unit and can give more information on the control of these processes.

Dedicated to S. Prakash Datta

2. MATERIALS AND METHODS

Albino male rats of the Sprague-Dawley strain, weighing 200–250 g, and fasted for 24 h, were used. Treatment of animals and exercise protocol were described previously [11]. The exercise consisted of running at 20 m per min on a horizontal treadmill; trained rats were subjected to 1 h of exercise, 7 days per week, for 1 month. Parenchymal cells were isolated from the rat livers immediately after 1 h of exercise in the case of the acute exercised rats and at the same hour in the case of controls. The isolated cells [13,14] were incubated as described by Krebs et al. [14]: the reaction was stopped by addition of 0.12 ml of 70% $HClO_4$ and glucose and citrulline concentrations were measured by the methods of Bergmeyer et al. [15]

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and of Nuzum and Snodgrass [16], respectively, in extracts neutralized with 20% KOH + 0.3 M Mops. Collagenase was purchased from Worthington. Enzymes used for glucose determination were from Boehringer Mannheim. All reagents were of analytical grade.

3. RESULTS AND DISCUSSION

It is well known that hormonal reassessment occurs during exercise. As soon as blood glucose begins to decline the levels of plasma glucagon, epinephrine and glucocorticoids increase [2], while that of insulin decreases [17]. There is much evidence that these modifications play a significant role in the control of gluconeogenesis, urea synthesis and mitochondrial respiration [18–20]. The data presented here indicate that the relationship between these processes may be very complex in situations created by different types of exercise. In a previous study we demonstrated that mitochondria prepared from livers of acutely exercised, untrained and trained rats exhibit an increased rate of respiration in state 3 [11]. Nevertheless, we have found no increase of glucose synthesis from different substrates including various amino acids following 1 h of exercise by untrained rats (fig.1 and table 1).

Glucose synthesis from glycerol was depressed significantly ($p < 0.05$) following acute exercise. There was a tendency for gluconeogenesis from some of the amino acids to be depressed but the differences were not significant. Valine and leucine led to no net synthesis of glucose.

It is possible that the relatively mild bout of exercise we employed did not alter hormonal levels sufficiently to enhance glucose synthesis. Winder et al. [21] found that plasma glucagon was increased only 25% in rats run for 45 min at 26 m/min up a 15% grade, and that plasma insulin, epinephrine and norepinephrine did not change. Furthermore, we found [11] that acute exercise did not cause a significant decrease of blood glucose concentration in untrained rats.

The rate of glucose synthesis from lactate was decreased in resting rats that had been subjected to a program of exercise training (fig.1). However, following a 1 h period of exercise, the rate was nearly doubled. Training did not influence the rate of gluconeogenesis from pyruvate in hepatocytes obtained in the resting state but again, an acute bout of exercise resulted in dramatic enhancement of glucose formation.

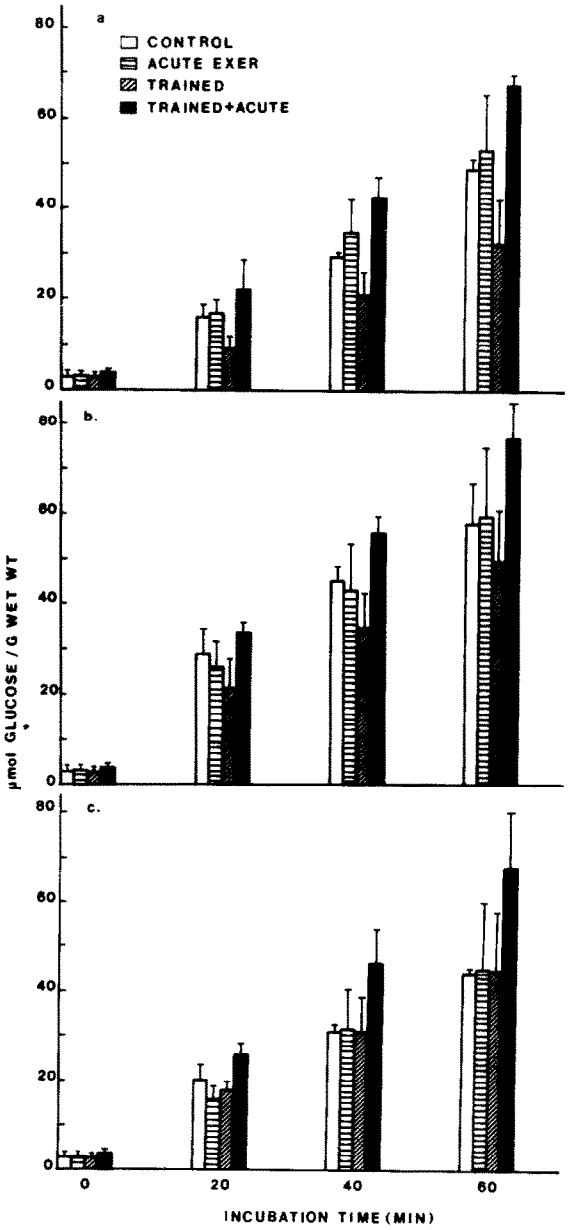

Fig.1. Effect of different types of exercise on gluconeogenesis in rat hepatocytes. The substrates were: (a) 10 mM lactate, (b) 10 mM lactate + 10 mM alanine, (c) 10 mM pyruvate. Columns represent means of 3–4 separate experiments. Vertical lines indicate SD.

The addition of alanine to lactate increased the rate of gluconeogenesis in all types of hepatocytes used and diminished the differences between trained and control rats (fig.1). It is worth mentioning that Clemens et al. [22] found a depressed gluconeogenic rate from lactate in hepatocytes from experimental septic rats, which was restored by addition of lysine. Unfortunately, they did not measure the glucose synthesis from pyruvate. These results together with observations of Cornell et al. [23] and Zahlten et al. [24] may indicate that long-term training affects the glutamate-aspartate shuttle which is preferentially used during gluconeogenesis from lactate.

The prolonged exercise training period did not affect the rate of citrulline accumulation (fig.2). Hepatocytes isolated from untrained rats following a bout of acute exercise produced somewhat more citrulline than cells from unexercised controls but the increase was not significant. In cells from exercise-trained rats the rate was clearly increased (fig.2) and correlated well with the increased rate of mitochondrial oxidation and phosphorylation [11]. It is likely that after the month of exercise training both catabolism of muscle protein and hepatic gluconeogenesis adapt so as to respond rapidly to the increased energy demand.

Table 1

Effect of acute exercise on glucose synthesis in rat liver parenchymal cells

Substrate (no. of experiments)	μmol glucose/g wet wt per min	
	Control	Acute exercise
None (7)	0.092 ± 0.028	0.095 ± 0.023
5 mM glycerol (8)	0.64 ± 0.194	0.48 ± 0.179
10 mM glutamine (7)	0.46 ± 0.177	0.37 ± 0.065
10 mM alanine (7)	0.47 ± 0.14	0.36 ± 0.118
10 mM isoleucine (4)	0.12 ± 0.006	0.094 ± 0.074
10 mM proline (6)	0.35 ± 0.087	0.27 ± 0.080
10 mM serine (4)	0.37 ± 0.086	0.35 ± 0.057
10 mM glycine (4)	0.13 ± 0.049	0.14 ± 0.054
10 mM asparagine (4)	0.37 ± 0.141	0.24 ± 0.081

Untrained rats were fasted for 24 h. Exercised rats ran for 1 h at 20 m/min. The preparation and incubation of the cells were as described in section 2. The cells were incubated for 40 min at 37°C in media equilibrated with 95% O_2 + 5% CO_2

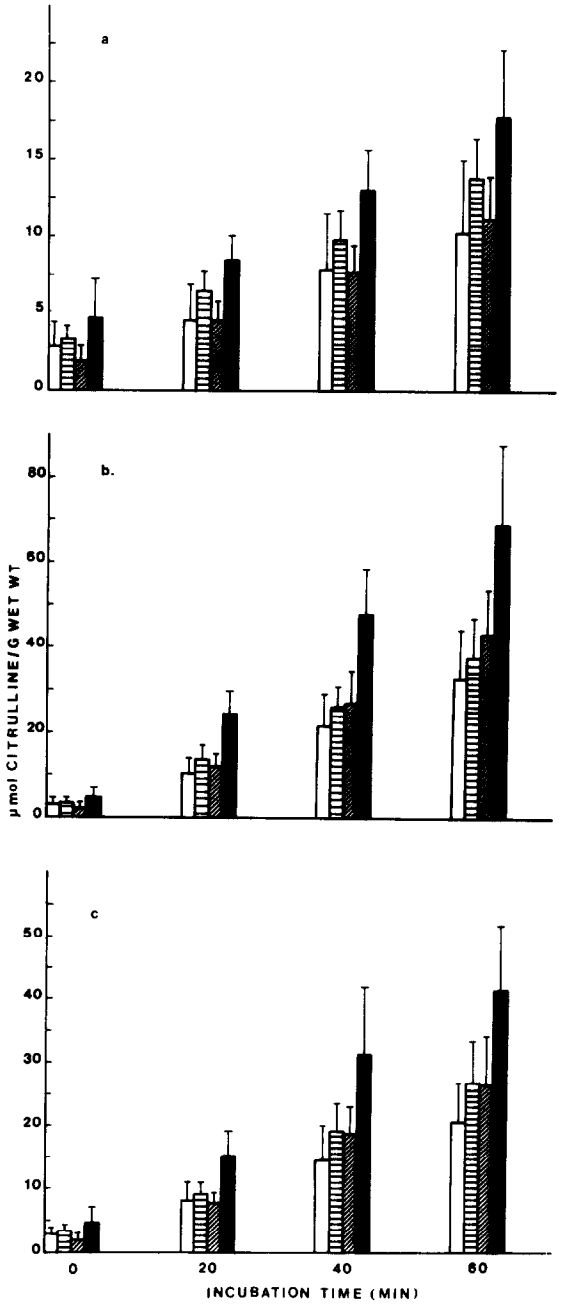

Fig.2. Effect of different types of exercise on citrulline synthesis in rat hepatocytes. The substrates were: (a) 10 mM lactate, (b) 10 mM lactate + 10 mM alanine, (c) 10 mM proline. Data are means ± SD of 3–5 separate experiments. The symbols indicating types of exercise are as in fig.1. The synthesis of citrulline in absence of substrates was at the same level as with lactate.

In conclusion, the present research data indicate that there is no correlation between mitochondrial respiration and gluconeogenic and urea synthesis processes in livers of acutely exercised untrained rats and in livers of trained animals. An increase of glucose and citrulline synthesis concomitantly with increase of mitochondrial respiration was observed only in the trained, 24 h fasted rats immediately following a bout of exercise. The present study along with that of French et al. [25] indicates that the model of isolated hepatocytes can be used for the study of different metabolic conditions created during exercise, because these cells retain during preparation at least some of the functional differences imprinted in vivo.

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