# EFFECT OF MUSCULAR EXERCISE AND GLYCOGEN DEPLETION ON RAT LIVER AND KIDNEY PHOSPHOENOLPYRUVATE CARBOXYKINASE

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## 1. Introduction

The effect of muscular exercise (physical training) on gluconeogenesis in rat kidney cortex has been reported by Krebs [1]. Forced exercise (swimming) increases the gluconeogenic capacity of the kidney, almost as much as a low-carbohydrate diet. On the other hand, Yakovlev has found an increase in the activity of rat liver glucose-6-phosphatase in similar conditions [2]. The well established key role of phosphoenolpyruvate carboxykinase (PEPCK) in gluconeogenesis and its sensitivity to hormonal treatment or starvation in liver and kidney [3–7] prompted us to investigate the effect of exercise on this enzyme.

## 2. Experimental

Female Wistar rats weighing 150–200 g were used. The animals were exercised by forcing them to swim in a warm water bath (22°) for 2 hr. The rats were sacrificed by cervical dislocation. The liver and slices of kidney cortex were homogenized with 10 vol. of 0.01 M Tris-HCl buffer, pH 7.4, containing 0.25 M sucrose. The homogenates were centrifuged at 35,000 *g* for 20 min. The PEPCK was assayed spectrophotometrically in the direction of oxalacetate synthesis in the presence of excess malate dehydrogenase at 30°. The assay mixture contained 100 μmoles Tris-chloride buffer, pH 7.4, 30 μmoles $MnCl_2$, 0.75 μmoles NADH, 30 μmoles phosphoenolpyruvate, 30 μmoles IDP, 2 μmoles reduced glutathione, 50 μmoles $NaHCO_3$, 0.05 ml particle-free supernatant and 50 μg of dialysed malate dehydrogenase in a total volume of 3.1 ml. Protein was determined by the method of Lowry et al. [8]. The glycogen determination was carried out as described by Krebs et al. [9]. The quantities of glycogen are expressed as glucose equivalents.

## 3. Results and discussion

The results are shown in table 1. Exercise alone has no effect on the liver PEPCK. Because of the dependence of PEPCK activity on glycogen content in liver [10–12], glycogen depletion was needed to test the effect of short-term exercise on the hepatic enzyme activity. Nicotinic acid has been shown to have an antilipolytic effect [13]. Following the injection of antilipolytic agents, unesterified fatty acid levels in the plasma decline. Consequently, more glucose is metabolized and liver glycogen stores are reduced [14] The intraperitoneal injection of nicotinic acid (500 mg/kg body weight) to well fed rats caused a decrease of liver glycogen content similar to that caused by fasting 48 hr. The PEPCK activity was enhanced to a similar extent in both conditions (160% of the control values). There was no further effect of short-term severe exercise on the enzymatic activity. These findings provide further evidence that the activity of PEPCK in liver is related to the depletion of the glycogen store rather than, directly, to the other physiological circumstances.

Nicotinic acid treatment was without effect on the renal PEPCK, although in starvation PEPCK activity of kidney cortex displayed an average 3.6-fold rise over normal values. The lack of effect of nicotinic acid treatment on the renal PEPCK was expected be-



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Table 1
Effect of exercise and glycogen depletion (starvation or nicotinic acid treatment) on phosphoenolpyruvate carboxykinase of rat liver and kidney cortex.

| Experimental conditions | Number of experiments | Liver glycogen * | Liver PEPCK ** | Kidney cortex PEPCK ** |
|---|---|---|---|---|
| Control (a) | 5 | 210 ± 42 | 21.4 ± 2.1 | 25.4 ± 3.1 |
| Exercise (2 hr) | 5 | 55 ± 13 | 20.2 ± 3.2 | 53.6 ± 3.9 |
| Control of nicotinic acid treatment (b) | 6 | 121 ± 21 | 26.3 ± 5.3 | 22.4 ± 3.9 |
| Nicotinic acid treatment | 6 | 14 ± 10 | 42.3 ± 1.0 | 24.3 ± 3.4 |
| Exercise and nicotinic acid treatment (c) | 6 | 10 ± 8 | 43.4 ± 10 | 48.2 ± 3.9 |
| Starvation (48 hr) | 6 | 8 ± 4 | 43.8 ± 4.2 | 86.9 ± 12.1 |

* Liver glycogen is expressed as glucose equivalents in μmoles/g of fresh liver weight.
** The PEPCK activity is expressed in nmoles of oxalacetate formed at 30° per min per mg of protein.
a) Starvation ( 2hr).
b) Controls were injected with saline and sacrificed after 7 hr. The animals were starved during this interval.
c) Well fed rats were forced to swim for 2 hr, 5 hr after nicotinic acid treatment (i.p. injection of 500 mg/kg body weight).

cause the quantities of glycogen in the kidney are very small [15]. On the other hand, the higher increase found in the renal enzyme of starved rats is in agreement with the fact that the renal cortex shows, per unit weight, greater adaptable changes favouring gluconeogenesis than the liver.

The enzymatic activity of kidney cortex extracts in exercised rats both before and after glycogen depletion was twice that of control animals. This enhancement was of the same order as the reported increase of the gluconeogenic capacity of rat kidney cortex slices caused by physical training [1], suggesting a relationship between both findings. The enhanced renal gluconeogenesis would be related to an increased activity of PEPCK.

A physiological condition in which the acceleration of renal gluconeogenesis is related to a higher PEPCK activity is metabolic acidosis [16–21]. In this situation there is an increased production of ammonia. It is very likely that the enhanced gluconeogenesis serves to remove glutamate and α-ketoglutarate and thereby favours ammoniogenesis. It has been well established that during strenuous physical exercise substantial quantities of lactate appear in the plasma and produce acidosis. Therefore, the changes in activity of renal PEPCK described in this paper may be brought out by similar mechanisms. Flores and Alleyne [22] have recently suggested that the early and rapid increase in PEPCK activity in response to metabolic acidosis is not due to a new synthesis of enzyme but to an activation of an inactive form of the enzyme already present. The enhancement in the renal PEPCK produced by the exercise is very quick (2 hr) and also suggests a mechanism of activation of the pre-existing form.

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