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Influence of Fasting and Hormone Deficiency on Myocardial Glycogen Levels in Rats[1] (38830)

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Cardiac glycogen metabolism has been the subject of intensive research for many years. In 1934, Evans (1) reported that fasting by experimental animals caused a significant increase (45–69%) in the glycogen levels of normal rat hearts. Since that time, other investigators (2–6) have studied the role of various hormones in this anabolic process and have found conflicting results. Part of these differences can be ascribed to either control procedures or experimental designs utilized. Therefore, we felt it necessary to reinvestigate aspects of this problem by comparing the effects of a variety of hormonal deficiencies on myocardial glycogen metabolism in the fed and fasted state of rats.

Methods. Adult male Sprague–Dawley rats were assigned to one of the following four experimental groups: normal control (NORM), hypophysectomized (HYPOX), adrenalectomized (ADX), and diabetic (DB). The HYPOX rats were purchased from Hormone Assay of Chicago, IL, whereas all others were purchased from Bio Lab of White Bear, MN. Bilateral adrenalectomy was accomplished by using the dorsal approach on rats under ether anesthesia. These animals were given 0.9% saline solution for drinking and allowed a minimum of 14 days recovery before being used for research purposes. The effectiveness of the surgical procedure was verified by the water test of Beatty (7) as described in an earlier report (8) and by inspection of the suprarenal area at the time of sacrifice. Experimental diabetes was induced by a single subcutaneous injection of alloxan monohydrate (4% w/v in saline) in a dose of 100–150 mg/kg. To enhance the alloxan effects, the animals were fasted 48 hr prior to injection. It should be noted that a 25–30% mortality rate was experienced with this injection protocol. The presence of diabetes was confirmed by determination of glucosuria (Clinistix) and blood glucose using either the anthrone technique (9) or the glucostat procedure (Worthington Biochemical Corporation). Plasma glucose levels for normal and diabetic animals were 128 ± 24 and 672 ± 78 mg/100 ml ($\bar{x}$ ± SE), respectively.

Animals from within each group were then assigned to either a fed or fasted subgroup. The fed animals were provided food and water ad lib. while the fasted animals were deprived of food for 30–36 hr with access to water. At the time of sacrifice, the animals were anesthetized with sodium pentobarbital (50 mg/kg ip) and the heart rapidly excised (5–10 sec) and placed in ice. While on ice, small left-ventricular samples were dissected, weighed and digested in hot 30% KOH. Ethanol (95%) was then added to precipitate the glycogen which was subsequently analyzed by the phenol–sulfuric acid method of Lo *et al.* (10). All animals were sacrificed in the morning hours between 7:00 and 11:00 AM to minimize the variability associated with biological rhythm (11).

Results. Cardiac glycogen concentrations of the various groups are summarized in Table I. It can be observed that fasting resulted in a significant (57%) increase in the concentration of glycogen in NORM animals and a significant (21%) decrease in HYPOX animals. In the fed state there were no differences between these two groups. However, fed ADX animals exhibited a 37% decrease in myocardial glycogen when compared to fed NORM animals. When the ADX animals were fasted, there was a significant (76%) increase in glycogen concentration. Table I also shows that the DB rats had significantly higher (55%) cardiac glycogen levels in the fed state than NORM-fed levels and that these levels were unchanged in the fasted condition.

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TABLE 1. CARDIAC GLYCOGEN LEVELS IN FED AND FASTED RATS WITH HORMONE DEFICIENCY.

Condition	Cardiac glycogen (mg/g)		%Δ
	Fed	Fasted	
Normal control	5.37 ± .18 (12)	8.44 ± .50 (7)[a]	57
Hypophysectomized	5.14 ± .26 (11)	4.04 ± .33 (8)[a,b,c]	21
Adrenalectomized	3.38 ± .20 (8)[a]	5.94 ± .45 (9)[b,d]	76
Diabetic	8.30 ± 1.06 (10)[a]	8.23 ± 1.25 (10)[a]	—

Means ± SE are listed. The number of animals per group is in parentheses. Denotes a significant difference from normal control fed[a], normal control fasted[b], hypophysectomized fed[c], and adrenelectomized fed[d] at $P < 0.05$.

Discussion. The effect of fasting on changes in the cardiac glycogen stores of normal animals is well documented (1, 3, 12, 13). However, controversy exists with respect to the hormonal influences on myocardial glycogen metabolism. The findings of Russell and Bloom (3) led them to conclude that adrenalectomy had no significant effect on myocardial glycogen levels from either fed or fasted animals. Daw *et al.* (5) as well as Poland and Trauner (6) reported that adrenalectomized animals had significantly lower cardiac glycogen levels than normal animals and concluded that glucocorticoids were essential for the regulation of myocardial glycogen. However, this finding is in contrast to the data of Poland and Trauner (6) who show in their Fig. 1 that cardiac glycogen in adrenalectomized animals was rapidly being resynthesized after depletion by exercise. Our findings in Table I demonstrate that adrenalectomized animals are capable of glycogen synthesis as evident from the 76% increase in substrate level after fasting. Consequently, we are postulating that glucocorticoids are important for the normal maintenance of resting cardiac glycogen; but, they have a limited role in the supercompensation effect associated with either fasting or exercise. Indeed, the data of Daw *et al.* (5) and Poland and Trauner (6) would support such a viewpoint.

The changes in myocardial glycogen levels with hypophysectomy or alloxan diabetes may be explained by substrate availability. Feeding a fatty meal to fasted HYPOX animals (4) or to normal animals (14) has been shown to cause an increase in myocardial glycogen levels similar to the increase associated with fasting by normal animals. This is a glycogen-sparing effect mediated by the increased uptake and utilization of free fatty acids by the myocardium (15). Similarly diabetes causes the myocardium to increase lipid utilization (16, 17) resulting in a concomitant rise in intracellular citrate levels which inhibit phosphofructokinase—the rate-limiting enzyme of glycolysis. This results in elevated glucose-6-phosphate concentration which can shift carbohydrate pathways toward glycogen formation and storage (16, 18). Therefore, the process of fasting would have minimal influence on existing levels of myocardial glycogen in diabetic rats. Our data in Table I would support such a concept.

Summary. This study was undertaken because of uncertainties regarding the influence of hormones on myocardial glycogen metabolism of fed and fasted rats. The results indicate that adrenal hormones exert a stabilizing effect on myocardial glycogen levels in fed animals but are not necessary for synthesis to occur. Hypophysectomy eliminates the glycogen increase that occurs from fasting in normal animals while insulin deficiency leads to elevated glycogen stores in both fed and fasted conditions. These findings suggest that changes in myocardial glycogen metabolism are the result of a synergetic relationship between a variety of hormonal and nutritional factors.

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