Effect of high lipid diet on circadian rhythms of tissue glycogen[1]

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Summary. The well defined circadian rhythms of glycogen content in heart, diaphragm and liver of the rat are drastically altered by a high lipid diet as shown by changes in amplitude, phase and tissue glycogen levels. If sampling times had been restricted to certain hours of the day the profound effect of the high fat diet on tissue glycogen would not have been apparent.

Physiological functions vary in a 24-h, or circadian, manner. These rhythms can be utilized to study metabolic interrelationships and to observe similarities or differences in 2 or more tissues under identical conditions. Circadian rhythms of glycogen content have been described in rat heart[3-5], rat diaphragm[5,6] and in liver glycogen[7,8]. The rat heart has both free fatty acid (FFA) and triglyceride (TG) rhythms which are 180° out of phase with the glycogen rhythm, while the diaphragm rhythms of glycogen and lipid have nearly simultaneous peaks and nadirs[5]. It is quite probable that these 2 continuously active muscles have different mechanisms for the control of the interaction of carbohydrate and lipid substrates. Studies of the effects of high lipid diets on liver glycogen have produced conflicting data[9,10] which may be due in part to circadian influences. TG levels in diaphragm[11,12] and heart and liver[13] have been found to increase in rats fed high fat diets. This study was designed to investigate possible differences between ventricle and diaphragm in the circadian utilization and/or storage of glycogen during increases in plasma FFA[10] and tissue TG[11,13] brought about by a lipid diet[10,11,13]. In addition, this study was designed to clarify the effect of a lipid diet on liver glycogen as examined through the use of circadian rhythms.

Male Wistar rats of about 100 g were entrained to a 12:12 light: dark cycle (lights on 06.00–18.00 h) in 25 °C animal room. Following 10 days of 1% Purina Lab Chow (approximate analysis, 10% fat, 22% protein and 68% carbohydrate) and water ad libitum, one-half of the rats were placed on a diet of pure semisolid lard, which they consumed readily. Control rats remained on the chow diet. After 5 days of the lard diet, animals were sampled at 4-h intervals to represent 6 time points in the 24-h cycle. Anesthesia was 40 mg/kg of sodium pentobarbital given i.p. Ventricles were rapidly excised through a ventral thoracotomy and frozen with liquid nitrogen-cooled clamps. 1 leaf of the diaphragm and the anterior margin of the liver were excised from the same animals and similarly frozen. The stomach was removed from each animal and the contents dried and weighed to provide a measure of food consumption. Glycogen analysis was conducted according to the anthrone method of Roe and Dailey[14], and the values are expressed as mg glycogen/g tissue wet weight. The data were statistically examined by the one-way analysis of variance and a p-value of less than 0.05 was regarded as evidence of a significant circadian rhythm.

Ventricular glycogen (figure 1) in the chow-fed rats showed a significant circadian rhythm with an acrophase at 04.00 h and a nadir at 16.00 h. Ventricles from the lard-fed rats also had a significant circadian rhythm with a distinct nadir at 08.00 h and a less distinct peak at about 12.00 h. At the 08.00 point, the chow-fed and lard-fed ventricular glycogens are not statistically different, but at all other time points, the ventricular glycogen levels of lard-fed rats are all higher than control levels. The circadian rhythms of diaphragm glycogen in chow-fed and lard-fed rats are

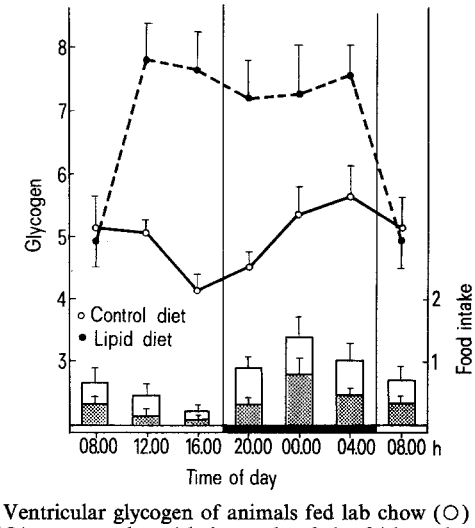

Fig. 1. Ventricular glycogen of animals fed lab chow (○) and lard diets (●), measured at 4-h intervals of the 24-h cycle. Stomach contents of chow- (□) and lard- (▨) fed animals were measured at the same times and expressed as g (dry weight)/100 g b.wt. Values shown at each time point are means ± SEM for 6–9 animals. Lights were on 06.00–18.00 h, and off 18.00–06.00 h.

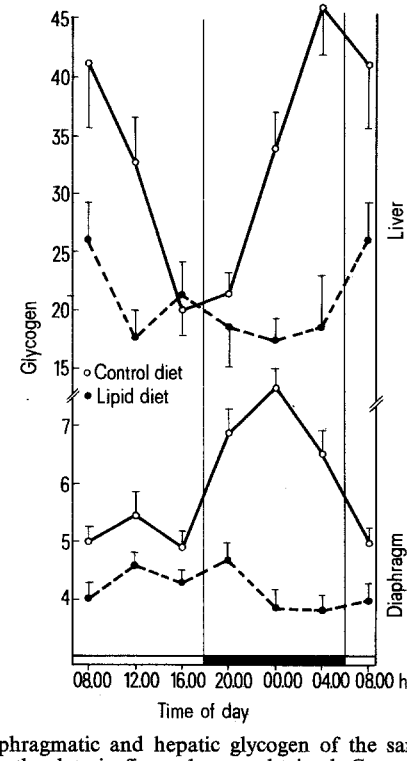

Fig. 2. Diaphragmatic and hepatic glycogen of the same animals from which the data in figure 1 were obtained. Control lab chow diet (○); lipid diet (●).

shown in figure 2. The diaphragmatic glycogen levels of lard-fed rats were much lower than those of control, and the 24-h fluctuation was much less dramatic. Also illustrated in figure 2 is the circadian rhythm of hepatic glycogen in chow-fed rats. Hepatic glycogen content in lard-fed rats diminished over most of the 24-h cycle, and was not statistically rhythmic. At 16.00 and 20.00 h, hepatic glycogen levels of chow-and lard-fed rats were not different.

The entrainment of feeding activity of both animal groups to the light: dark cycle is apparent from the stomach contents data shown in figure 1. Although the feeding patterns of chow-fed and lard-fed rats are identical, there are differences in the times of the glycogen peaks and nadirs between the 2 diet groups and among the 3 tissues. The volume of food ingested by the lard-fed rats was lower than controls at all times sampled due to the low bulk and high caloric content of the high lipid diet.

This study confirms a differential effect of a lipid diet on ventricular and diaphragmatic glycogen rhythms and indicates a difference in metabolic control mechanisms between these 2 types of continuously active muscles. The increase in ventricular glycogen in lipid-fed rats presumably results from an acceleration of fatty acid oxidation which causes an inhibition of glycolysis at the phosphofructokinase[15] and pyruvate dehydrogenase[16] enzymes. The production of $^{14}CO_2$ from labeled pyruvate and pyruvate uptake have been shown to decline in diaphragms of rats fed high fat diets, even though acetate oxidation was unchanged, implying a blockage of glycolysis at pyruvate dehydrogenase[10,11]. There seems to be no evidence of a similar blockage at phosphofructokinase in diaphragm with lipid feeding. Diaphragm, unlike heart, does not have significant glycerol kinase activity[17], and utilizes the glycolytic production of dihydroxyacetone-phosphate to provide glycerol phosphate for the synthesis of triglyceride. The inhibition of glycolysis at pyruvate dehydrogenase, but not at phosphofructokinase, would allow the formation of dihydroxyacetonephosphate as a source of glycerol-phosphate, and help to explain a decline in diaphragm glycogen in response to a diet that is known to increase diaphragm triglycerides[11].

The depletion of liver glycogen in the lard-fed rats is consistent with the role of the liver in the maintenance of blood glucose during carbohydrate deprivation. However, it has been reported that rat liver phosphorylase is inhibited by feeding a fat diet[18], and the decline in glycogen levels may be due as well to a decrease in glycogen synthesis. Without cyclic fluctuations in the carbohydrate delivered to the liver from absorbed nutrients, the rhythmicity of liver glycogen is lost or significantly dampered. It is apparent from this study that the 24-h variation in liver glycogen in control rats is quite dramatic.

The comparison of ventricular and hepatic glycogen in chow-fed and lard-fed rats would have yielded no differences in tissue levels if sampling were limited to certain times in the circadian cycle. This point is germane to any study of metabolism in tissues which have substrate rhythms.

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Behavioral fever induced in guinea-pigs by intrapreoptic pyrogen[1]

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Summary. The preoptic area of the hypothalamus integrates not only the autonomic, but also the behavioral components of fever in guinea-pigs.

It is now generally recognized that behavioral adjustments are an integral part of physiological temperature regulation. It is not surprising, therefore, that behavioral responses also are normal adjuncts of fever production and lysis in all classes of vertebrates, from fishes to mammals[2]. A commonplace example among mammals is man in the first stages of fever reporting feeling cold, seeking increased insulation and reducing his heat-exchanging surface area in order to warm himself. Fever also has a great influence on thermal preference. Thus, fishes and amphibians actively seek higher ambient temperatures following injections of pathogenic organisms; and, in operant selection of thermal reinforcers experiments, cats, dogs, and baboons injected systemically with pyrogens respond with an increased rate of lever-pressing for external heat during the phase of rising temperature.

It has been established that the preoptic-anterior hypothalamic (POAH) region is involved in the control of behavioral thermoregulation[3]. Thus, warming this area elicits a decrease in behavioral heat-acquisition responses, an increase in behavioral heat-escape responses, and a lowering of an animal's preferred ambient temperature; cooling this area produces the opposite effects. That the POAH also is the locus of greatest control of autonomic thermoregulatory responses has been repeatedly shown[4]. Since endogenous pyrogen (EP) also localizes in this region to trigger the total