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Circadian Rhythms of Glycogen, Free Fatty Acids, and Triglycerides in Rat Heart and Diaphragm (40458)

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Variations in glycogen content over a 24-hr period have been described in rat heart (1, 2) and in several rat skeletal muscles (3, 4), including diaphragm (4). The relationship of the circadian rhythm in glycogen content to muscle tissue levels of other endogenous substrates has not been reported. The heart and diaphragm are two different muscles which must be active throughout the entire day in order to sustain life. Examination of cyclic variations of free fatty acids (FFA), triglycerides (TG) and glycogen in these tissues, in the absence of any stress to the animal, can provide information about *in vivo* metabolism and its control, and illuminate differences that may exist between cardiac and diaphragmatic utilization and storage of these substrates.

This study was undertaken to determine if there are circadian rhythms of FFA and TG in the rat heart and diaphragm, and if so, what phase relationships the lipid rhythms bear to the glycogen rhythm.

Materials and methods. Animals. To entrain the glycogen rhythm, male Wistar rats (150–300 g) were housed three to a cage in two animal rooms with 12:12 light–dark cycles. One room was 180° out of light phase with the other in order to facilitate simultaneous sampling of two time points 12 hr apart in the light cycle. Purina Rat Chow and tap water were available *ad libitum*. A minimum of disturbance of the animals was maintained throughout the 2-week entrainment period. Twelve animals per time point were utilized for determination of heart substrates, and six animals per time point were used for the diaphragm analyses.

Sampling and tissue analysis. There were six different sampling times 4 hr apart, three during the light period (0600–1800) and three during the dark period (1800–0600 hr). In the diaphragm analyses, the sampling times were 2 hr earlier than the sampling times which were used for the heart analyses. At sampling time, the animals were weighed and anesthetized with sodium pentobarbital, 40 mg/kg ip. Hearts were excised through a ventral thoracotomy and frozen within 5 sec with liquid nitrogen-cooled clamps. The pericardium was removed during excision. Hearts were free of any visible surface adipose deposition, so no attempt was made to remove any epicardial tissue or surface vessels. The great vessels were cut away at the base of the heart. Both leaves of the diaphragm were removed from the second group of rats. Using a scalpel, phrenic nerve, clinging fatty tissue, central tendon, and chest wall attachments were all rapidly stripped from the diaphragm samples. The connective tissue sheath of the diaphragm remained otherwise intact. Diaphragms were also frozen with liquid nitrogen-cooled clamps. All tissue samples were matted on paper toweling to minimize blood in the samples. Stomachs of all animals were excised and the contents weighed to approximate food consumption. Muscle glycogen content was determined by the anthrone method (5). Following extraction of tissue lipids (6), TG levels (7) and FFA content (8) were measured. Phospholipids were removed from the tissue extracts as part of the triglyceride analysis (7).

Statistical analysis. The data were examined statistically by analysis of variance and a p-value of 0.05 or less was regarded as evidence that a significant circadian rhythm was present.

Results. Heart. Significant circadian variations were found in the levels of heart glycogen, FFA and TG (Fig. 1). The heart glycogen acrophase occurred at 0600 hr and the nadir was 1800 hr. The FFA and TG rhythms were 180° out of phase with the glycogen rhythm, with peaks occurring at 1800 hr and nadirs occurring at 0600 hr. Stomach contents were lowest at 1400 hr, then rose with the increase in feeding activity during the dark period of the cycle. Assuming a lag in food

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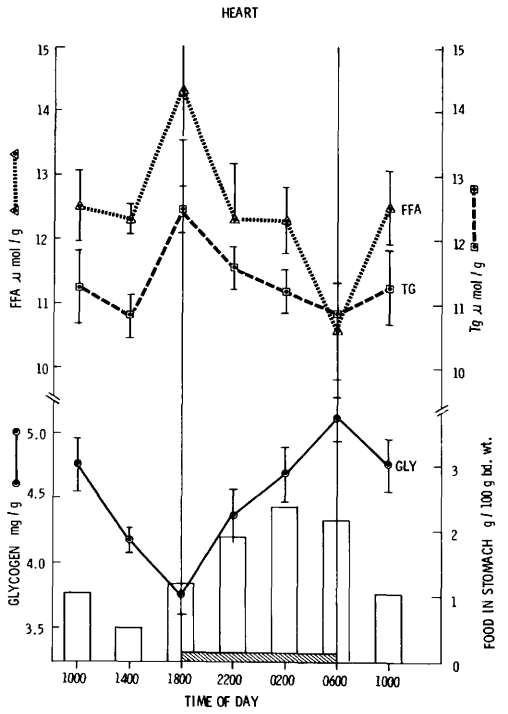

FIG. 1. Circadian rhythms of rat heart glycogen, free fatty acids and triglycerides, and 24 hr pattern of stomach contents. Dark period of the cycle is indicated by the shaded area between the vertical lines. Data shown are means ± SEM of 11–12 animals per time point, with significance of at least $p < 0.05$ for all three rhythms. Units are expressed per g of tissue wet weight, except food in the stomach, which is per 100 g body weight.

absorption, these data suggest that the rat myocardium stores lipid and depletes glycogen during reduced food ingestion, and depletes lipid and stores glycogen during times of maximal food intake. The glycogen and lipid rhythms were both entrained to the light–dark cycle.

Diaphragm. Circadian rhythms were also found to be present in diaphragmatic glycogen, FFA and TG (Fig. 2). The diaphragm glycogen peak occurred earlier than that of heart, the acrophase being 4–6 hr before the lights came on. In contrast to the heart substrate rhythms, the diaphragm FFA and TG peaks related more closely to the glycogen peak. Unlike heart, the diaphragm glycogen and lipid rhythms corresponded generally to the feeding pattern of the rat, with nadirs occurring at times of decreased feeding activity. The diaphragm substrate rhythms were also entrained to the light–dark cycle.

Examination of the 24 hr means and the peak and nadir values (Table I) of glycogen, FFA and TG indicates the higher mean levels and greater fluctuations of each substrate in diaphragm compared to heart over the 24-hr period.

Discussion. Circadian rhythms of endogenous glycogen, FFA and TG were found to be present in rat heart and diaphragm and were entrained to a 12:12 light–dark cycle. These rhythms are of a magnitude that warrants consideration of time of day when metabolic substrate measurements are made. The glycogen and lipid rhythms may be due to synchronized cellular-level oscillations in metabolic events which are independent of exogenous drivers, or they may be the manifestation of extracellular forces, but it is apparent that glycogen and lipid are mobilized in rat heart and diaphragm in the absence of

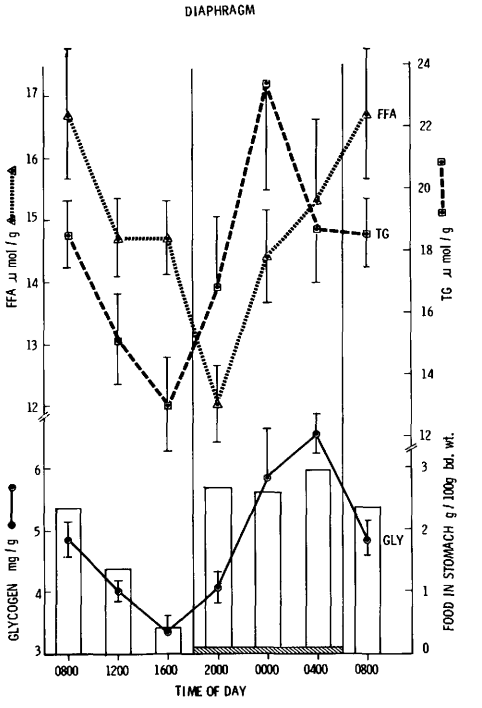

FIG. 2. Circadian rhythms of rat diaphragm glycogen, free fatty acids and triglycerides, and 24 hr pattern of stomach contents. Dark period of the cycle is indicated by the shaded area between the vertical lines. Data shown are means ± SEM of five to six animals per time point, with significance of at least $p < 0.05$ for all three rhythms. Units are expressed per g of tissue wet weight, except food in the stomach, which is per 100 g body weight.

TABLE I. 24 HR MEANS WITH ACROPHASES AND NADIRS.

		24 hr mean[a]	Acrophase[b]	Nadir[b]
Heart ($n = 72$)	Glycogen (mg/g)[a]	4.46 ± 0.23	5.11 ± 0.17	3.75 ± 0.14
	FFA (μMole/g)	12.38 ± 0.06	14.30 ± 0.80	10.60 ± 0.76
	TG (μMole/g)	11.38 ± 0.03	12.64 ± 0.37	10.81 ± 0.32
Diaphragm ($n = 36$)	Glycogen (mg/g)	4.73 ± 0.81	6.55 ± 0.32	3.38 ± 0.23
	FFA (μMole/g)	14.70 ± 0.10	16.70 ± 1.05	12.10 ± 0.61
	TG (μMole/g)	17.60 ± 0.22	23.4 ± 3.52	13.0 ± 1.57

[a] Units in this column are expressed per g tissue wet weight.
[b] Values in these columns are Mean ± SEM.

stress or hypoxia. Despite exposure to the same internal environment, these two muscles exhibit different patterns in the utilization of glycogen and lipid. Glycogen is depleted as lipid is stored in heart, while diaphragm utilizes or stores these substrates nearly simultaneously. Circadian rhythm studies which make use of these continuously active muscles provide a unique model for elucidating the characteristics of *in vivo* metabolism of cardiac and skeletal muscles. Such studies are conducted without altering the real microenvironment of these muscle cells; i.e. they illuminate the results of the metabolic regulation that occurs naturally at the existing ionic, substrate, and enzyme concentrations and interactions, with time and light as the only forcings.

It is likely that many interrelated factors contribute to the control of the glycogen, FFA and TG rhythms in heart and diaphragm, and that the controlling factors are different in the skeletal and cardiac muscle types. It has been reported that in contrast to perfused rat heart, in the isolated rat diaphragm a wide range of concentrations of fatty acids does not inhibit glucose transport or phosphorylation, or affect its conversion to glycogen (9). Gastrocnemius has been found to be less responsive than heart to the citrate-increasing effects of fatty acids (10). The oxidation of palmitate did not affect glycolysis in monkey sartorius muscle (11). These observations, coupled with the synchrony of the diaphragm substrate rhythms seen in this study, are consistent with the hypothesis that the inhibition of glycolysis by fat oxidation may not be as potent in skeletal muscle as it is in cardiac muscle. The degree to which fatty acid oxidation diminishes glycolysis may relate to the rate of glycolysis characteristic of the tissue (12), or to the muscle fiber type (13). Consistent with differences between diaphragm and heart metabolism are the observations that diaphragm FFA vary independently of plasma FFA (14), while heart FFA uptake and utilization tend to be proportional to plasma FFA concentration (15). The 180° phase difference in the FFA and TG rhythms of the heart compared to the diaphragm suggests little contribution of trapped plasma to the tissue rhythms.

Control of these rhythms by feeding activity seems unlikely. Heart glycogen is seen to rise during the nocturnal increase in feeding activity, although it is known that glycogen will increase in hearts of fasted rats (16). It has also been found that oscillations occur in heart and diaphragm lipoprotein lipase activity which are independent of feeding stimuli (17). The phase difference of heart and diaphragm glycogen rhythms found in this study and in another study (18) also tends to preclude control of the rhythms by feeding activity.

The findings reported here with regard to the heart are consistent with other studies (19) which find that endogenous glycogen is mobilized by rat heart even in oxygenated states. In heart, where the glycogen and lipid rhythms are out of phase, it could be postulated that glycogen degradation provides dihydroxyacetone phosphate for the glycerol portion of TG synthesis. The concurrent use of glycogen and lipid substrates, as in diaphragm, could ensure the provision of oxaloacetate for condensation with acetyl CoA in the Krebs cycle (20).

The absolute levels of tissue TG reported here are higher than those reported by some authors (21) and lower than those reported by others (22). These data, however, are in

good agreement with data reported elsewhere (23). The discrepancies in the literature may be due to the species, age and handling of the rats, the method of TG determination, and the purity and type of triglyceride standard utilized.

Summary. Circadian rhythms entrainable to a 12:12 light-dark cycle have been found in rat heart and diaphragm glycogen, FFA and TG. While the diaphragm rhythms of glycogen and lipid exhibit nearly simultaneous peaks and nadirs, the heart substrate rhythms are 180° out of phase. The two continuously active muscle types apparently have different control mechanisms for the metabolism and storage of endogenous substrates. It is possible that these rhythms reflect different metabolic functions in the two muscles. Circadian studies of heart and diaphragm metabolism provide a useful model for *in vivo* substrate utilization in the normal, unstressed animal.

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