# Endogenous Triglyceride and Glycogen in Perfused Rat Hearts (37365)

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Nonworking (1) and working (2) rat hearts perfused *in vitro* have been useful models for biochemical and physiological studies of cardiac function. The heart can utilize a variety of circulating metabolites for energy production and contractility, and under certain specific perfusion conditions, endogenous substrates might also be available as energy sources (3). The utilization of endogenous glycogen has been studied extensively (see Ref. (3)). However, based on observations that hearts depleted of glycogen will continue to contract and produce $CO_2$, Shipp *et al.* (4) suggested that endogenous lipids were used for energy by nonworking, perfused hearts. These workers reported that utilization of phospholipid fatty acids could account for 75% of total carbon dioxide production by the isolated heart during a one-hour perfusion. However, Olsen and Hoeschen (5) and Denton and Randle (6) have reported that during heart perfusion, only endogenous triglycerides are extensively mobilized for energy purposes. Kako and Dubuc (7) reported a significant decrease in cardiac phospholipids during perfusion of hearts from fasted animals, whereas, no decrease in this lipid fraction was found using hearts from fed rats.

In the present study, depletion of cardiac glycogen and lipids were determined simultaneously after perfusion of rat heart with Krebs bicarbonate buffer alone, or buffer containing epinephrine, dibutyryl cyclic adenosine monophosphate (dibutyryl cAMP), heparin, glucose, or albumin-bound palmitic acid.

*Methods. Perfusion conditions.* The apparatus for recirculating perfusion of rat hearts, handling of animals, and procedures for coronary perfusion have been described earlier (8). Male albino rats (Wistar strain, Wenonah Farms), weighing between 150 and 200 g, were fed *ad libitum* prior to use. They were anesthetized by intraperitoneal injection of sodium pentabarbital (25 mg/kg), hearts were rapidly removed from the animal and placed in preoxygenated Krebs bicarbonate buffer, pH 7.4 at 37°. Care was used to remove all extraneous adipose tissue from the area of the atria and aorta. A 16-gauge perfusion cannula was inserted into the aorta, tied in place, and the coronaries were perfused slowly with 10 ml buffer to remove blood and restore the heart beat. The heart and cannula were than transferred to the perfusion apparatus and the perfusion carried out for 15, 45, or 90 min during continuous gassing with $O_2$–$CO_2$ (95.5, v/v). The initial perfusate volume was 20 ml and perfusion pressure was maintained at about 60 mm Hg. Heart rates and coronary flow rates were monitored during all perfusions.

*Glycogen determination* (9). Immediately after perfusion, hearts were frozen on powdered dry ice. The tissue was pulverized, a portion (200 mg) was homogenized in 10 ml ice-cold water and aliquots were hydrolyzed with KOH (5 *N*). After cooling, glycogen was precipitated overnight with 95% ethanol containing 0.1% lithium bromide. The precipitated glycogen was washed with 80% methanol containing 0.1% lithium bromide, glycogen was dissolved in water, and measured as glucose equivalents by the anthrone procedure (9).

*Extraction and analysis of myocardial lipids.* A weighed portion (0.4 g) of the frozen pulverized tissue was homogenized in 20 volumes of chloroform–methanol (2:1) to which was added palmitic acid-1-$^{14}$C (0.5 $\mu$Ci). Lipid extraction was carried out

according to the method of Folch *et al.* (10), and the radioactive palmitate was used as internal standard for total recovery of myocardial lipid during extraction and separation. Procedures for separation of major lipid classes, transmethylation and gas–liquid chromatography of fatty acids, determination of recoveries, and spectrophotometric analyses of glyceride glycerol have been reported earlier (11).

*Results. Substrate-free perfusions.* Nonperfused hearts from *ad libitum* fed rats contained 14.4 ± 1.0 μmole glycogen glucose and 57 ± 3.0 μmole total fatty acids/g wet weight tissue. Phosphatide fatty acids represented approximately 85%, and triglyceride fatty acids nearly 9% of the total fatty acids in cardiac tissue from which adipose tissue had been carefully dissected.

As shown in Fig. 1, during the initial 15 min of perfusion with Krebs bicarbonate buffer, cardiac glycogen decreased from 14.4 μmole/g to 4.8 μmole/g wet weight (33% of control), and no further significant decrease occurred with continued perfusion until 45 min. However, by 90 min of perfusion, at which time cardiac rate and coronary flow were drastically reduced (exhaustion), cardiac glycogen represented only 17% of the control level.

There was no significant depletion of total cardiac fatty acids after 45 and 90 min of buffer perfusion (57 ± 3 μmole for controls; 54 ± 6 μmole at 45 min; 50 ± 5 μmole at 90 min). This was due primarily to the fact that phosphatide fatty acids, which make up the bulk of the total cardiac fatty acids, were not altered by perfusion with buffer for 45 min (Fig. 1). However, a small but significant decrease in phosphatide fatty acids was observed at 90 min of perfusion in the absence of circulating substrates. In contrast, triglyceride fatty acids were significantly decreased by 24% in 45 min and 62% after 90 min of perfusion (Fig. 1).

Analysis of the fatty acid distribution of cardiac triglycerides in control and buffer-perfused hearts (Fig. 2) showed that there was no preferential utilization of any particular fatty acid during the period when

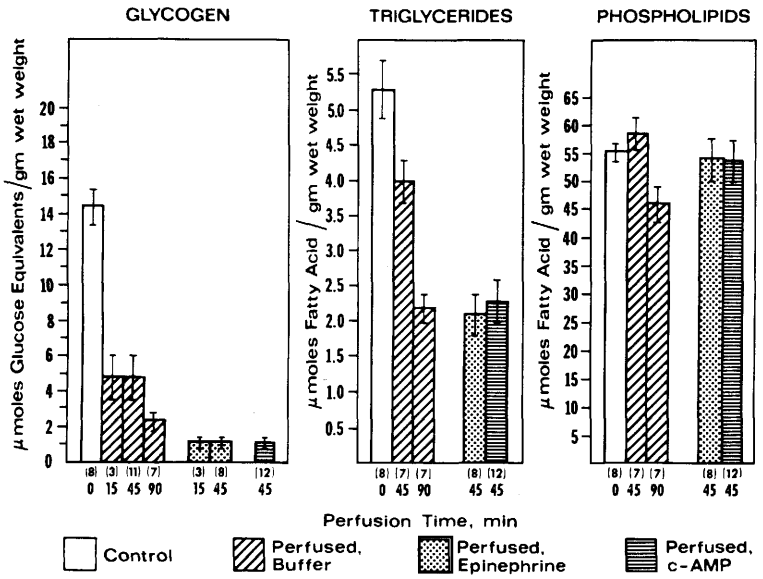


FIG. 1. Effect of perfusion with buffer alone, with $10^{-5}$ *M* epinephrine of $10^{-3}$ *M* dibutyryl cAMP on the levels of endogenous lipid and glycogen in isolated hearts. The lipid data are expressed as μmoles of fatty acid per gram wet weight of tissue ± SEM. Myocardial glycogen is expressed as μmoles glucose equivalents per gram wet weight of tissue ± SEM. The figures in parentheses indicate the number of determinations. $*p < 0.05$ and $**p < 0.01$ vs unperfused, control hearts.

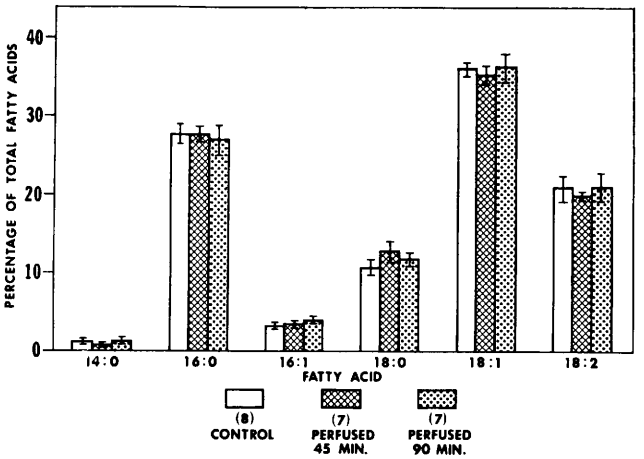


FIG. 2. Effect of perfusion of rat hearts with buffer for 45 and 90 min on the distribution of triglyceride fatty acids in isolated rat heart. Fatty acid composition of the triglyceride fraction is expressed as mole % ± SEM. The figures in parentheses indicate the number of determinations.

over half of cardiac triglycerides were mobilized.

*Perfusions with epinephrine or dibutyryl cAMP.* Perfusion of rat heart with $10^{-5}$ *M* epinephrine or $10^{-3}$ *M* dibutyryl cAMP resulted in identical effects on cardiac glycogen and triglyceride. With epinephrine, there was almost complete depletion of cardiac glycogen in 15 min (Fig. 1), and although heart rate and coronary flow were maintained, there was no further change in cardiac glycogen at 45 min of perfusion.

Epinephrine or dibutyryl cAMP perfusions also resulted in marked depletion of cardiac triglyceride but not phospholipid (Fig. 1). After 45 min, cardiac triglyceride was reduced to 2.1 μmole/g wet weight or approximately half of the triglyceride level in hearts perfused to exhaustion with Krebs buffer.

*Perfusion with substrates or heparin.* Recirculating perfusion with 8.3 m*M* glucose resulted in significantly less glycogen loss than perfusion with buffer alone (Fig. 3), and completely prevented the depletion of cardiac triglyceride observed during substrate-free perfusions. Results with palmitic acid perfusions were highly variable, and the levels of cardiac glycogen and triglyceride were not significantly different from the levels in unperfused controls or following glucose perfusion. In all cases of substrate perfusion, there were no significant changes in cardiac phospholipid levels.

Perfusions with heparin (1 mg/20 ml perfusate) did not prevent the marked depletion of cardiac glycogen observed during perfusion with buffer alone; however, there was significantly less loss in cardiac triglycerides than during buffer perfusion (Fig. 2) or perfusions with epinephrine and dibutyryl cAMP (Fig. 1).

*Discussion.* Our current knowledge of the relative importance of various circulating substrates as fuel for myocardial energy production has recently been extensively reviewed (3). It appears that under *in vivo* conditions, the heart utilizes primarily circulating glucose and free fatty acids or ketone bodies under specific conditions. The isolated heart preparation has been used extensively to determine various aspects of substrate uptake, metabolic pathways and metabolic control, but the applicability of this information to cardiac function *in vivo* is questionable (3). Nevertheless, it has been shown that endogenous substrates are capable of supplying fuel for energy metabolism and contractility of heart *in vitro* even when circulating substrates are available (12–14). In the present study, alterations in endogenous glycogen, triglyceride and phospholipid have been measured simultaneously under a variety of perfusion conditions *in vitro* and an attempt was made to determine the relation-

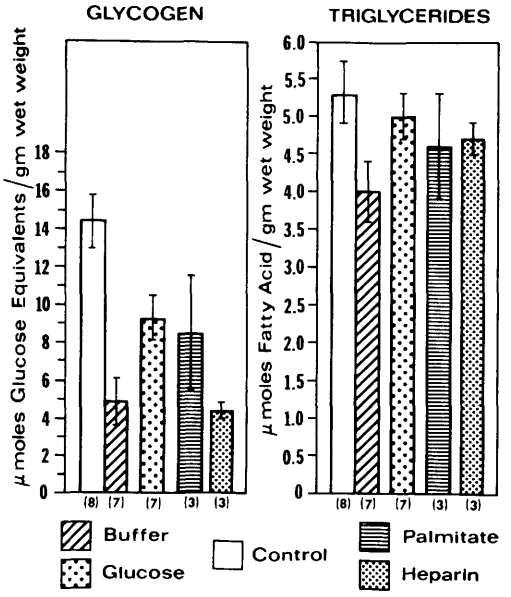


FIG. 3. Effect of 45-min perfusion of rat hearts with exogenous substrates on the levels of endogenous glycogen and triglyceride. The endogenous triglyceride results are expressed as μmoles of fatty acid present in the triglyceride fraction per gram wet weight of tissue ± SEM. Myocardial glycogen data are expressed as mole glucose equivalents per gram wet weight tissue ± SEM. The figures in parentheses indicate the number of determinations. $*p < 0.05$ and $**p < 0.01$ vs control, unperfused hearts.

ships of utilization of three potential energy sources rather than separately measuring utilization of either glycogen (15) or lipid (4–7). As has been shown earlier (15), cardiac glycogen is used rapidly by the perfused rat heart as an energy source to maintain cardiac contractility when circulating substrates are not available. This depletion is further exaggerated during perfusions with epinephrine or dibutyryl cyclic AMP. Despite the suggestion that cardiac glycogen is only mobilized during anoxia, the present data show that this energy reserve can be almost entirely depleted during oxygenation to maintain mechanical performance of the heart.

Cardiac triglycerides are also mobilized during substrate-free perfusions, although the extent of depletion of this fuel was not as dramatic as with glycogen. Even with perfusions of 90 min or "exhaustion" (5), during which cardiac contractions and coronary flow were markedly reduced, over 40% of the initial triglyceride level still remained in the tissue. Only at this time was it possible to show a small but significant decrease in cardiac phospholipids. These results are in general agreement with both those of Olsen and Hoeschen (5) and Denton and Randle (6) who reported the marked mobilization of cardiac triglyceride as lipid fuel for energy purposes, but could demonstrate no change in cardiac phospholipids, and of Kako and Dubuk (7), who indicated that phospholipids could be mobilized only after 60 min of substrate-free perfusions of hearts from fasted animals. Thus, it appears that phospholipid may be primarily a structural lipid in heart (5, 6) and its depletion after prolonged perfusion *in vitro* may reflect the loss in structural integrity of a failing heart preparation.

There were no significant changes in the fatty acid profile of cardiac triglycerides during substrate-free perfusion, even after 90 min when cardiac triglycerides had been depleted by almost 60%. Thus, there appears to be no preferential utilization of a specific fatty acid during lipolysis of endogenous triglycerides.

Perfusions with epinephrine or dibutyryl cAMP resulted in an increase in lipolysis of endogenous triglycerides resulting in triglyceride levels similar to that found in the "exhausted" heart (90 min, substrate-free perfusion). It has been reported that epinephrine perfusion produces a 3- to 4-fold increase in glycerol release from heart into the perfusion medium (16–18). Although these data and the present studies suggest the presence of a hormone-sensitive lipase similar to that in adipose tissue (19), it is also possible that increased utilization of triglyceride as an energy source is a direct result of the marked depletion of cardiac glycogen during the perfusion of the hormone or "second messenger" (see Fig. 1). It has not been possible to show a direct effect of cAMP on cardiac lipase *in vitro* (11).

Although heparin is known to increase the hydrolysis of circulating lipoprotein triglyceride catalyzed by lipoprotein lipase (19), the addition of heparin did not increase the

hydrolysis of intracellular triglyceride, but is only responsible for the cellular uptake of circulating triglyceride fatty acids.

*Summary.* Mobilization of endogenous lipid and glycogen by the isolated rat heart *in vitro* has been studied under a variety of conditions. Recirculating perfusion of hearts with Krebs bicarbonate buffer (45 min) led to a marked depletion (66%) of cardiac glycogen, while cardiac triglyceride was decreased by 24%. There were no changes in the levels of phospholipid, diglyceride, cholesterol ester, or free fatty acid. After perfusion for 90 min, in the absence of added substrates, endogenous glycogen and triglyceride were depleted to 16% and 38%, respectively, of the levels in the unperfused controls. During this second 45-min period of perfusion, a small but significant depletion of phospholipid also occurred, indicating that this lipid class is only used for energy after marked depletion of the other endogenous energy sources.

Perfusions with epinephrine ($10^{-5}$ *M*) or dibutyryl adenosine 3′,5′-monophosphate (dibutyryl-cAMP) for 45 min produced marked decreases in the levels of cardiac glycogen and triglyceride, but under these conditions, there was no apparent utilization of phospholipid fatty acids.

The depletion of cardiac glycogen during perfusion with buffer alone (45 min) was partially prevented, and depletion of cardiac triglyceride was completely abolished when glucose (9.2 m*M*) or albumin-bound palmitate (0.5 m*M*) were added to the perfusate. Perfusion with heparin resulted in a decrease in cardiac glycogen but did not affect triglycerides. The results suggest that cardiac triglycerides provide a secondary endogenous source of energy to the heart *in vitro*, only after marked depletion of glycogen; phospholipid fatty acids may be utilized following exhaustion of these other endogenous energy sources.

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