In vivo sampling of cardiac triglyceride from dogs during ethanol infusion

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Abstract The feasibility of procuring and analyzing cardiac tissue for triglyceride in vivo was tested in anesthetized dogs. Measurements of triglycerides in samples obtained in vitro confirmed: reproducibility of triplicate analyses of the glyceride-glycerol moiety of tissue triglyceride (SEM ± 2.1%), homogeneity in and between ventricles (SEM ± 1.8%), and agreement between right endocardial triglyceride and left myocardial triglyceride (difference not significant). Seven dogs received ethanol, 15–30 mg/kg/min, and five dogs received glucose or 0.85% NaCl for 2 hr. Cardiac output and filling pressure were measured from the left ventricle and tissue was taken from the right ventricle with a biopsy catheter before and during infusions. Three to four samples were obtained from each dog; the average weight was 14.4 mg and two to three biopsies were required for each sample. In the ethanol group, triglyceride increased after 15 min and continued to rise; the final triglyceride concentration correlated with the infusion rate. In the glucose–saline group, in vivo triglyceride concentration did not change and did not differ from postmortem triglyceride. Cardiac function declined in the ethanol group and was unaffected in the controls. Thus, multiple in vivo measurements of cardiac lipid are practical and safe and show that ethanol infusions cause early and progressive accumulation of triglyceride in heart muscle.

Supplementary key words cardiac biopsy · cardiac performance · autonomic blockade · propranolol · atropine

Metabolic studies in both the isolated and the intact heart have established that plasma free fatty acids are the preferred substrate for the beating heart. These experiments were reviewed by Opie in 1968–69 (1, 2). More recent investigations in intact subjects suggest that myocardial triglyceride is also burned as fuel and that this endogenous fat contributes to the plasma pool of lipids (3–8). Up to now, the technical difficulty of obtaining serial measurements of tissue fat has limited examination of the relative importance of myocardial lipid in cardiac metabolism.

The availability of a biopsy catheter makes it feasible to procure cardiac tissue in vivo for analysis. In these experiments, endocardial biopsies were collected for TG determination, and cardiac function was assessed before and during the constant infusion of ethanol. Alcohol was used because it is known to produce TG accumulation in the heart within hours (9). We attempted to answer the following questions. Is it possible to measure TG content from small samples of endocardium? Are these TG determinations representative of the TG concentration in both ventricles? Do multiple biopsies affect cardiac performance?

MATERIALS AND METHODS

22 mongrel dogs of both sexes were used; 12 of these (experiments 11–22) weighing 20.1–31 kg were the subjects of the combined metabolic and hemodynamic study. All of the animals were housed at our animal facility for at least 3 wk before the study, and they were fed commercially prepared dry dog food (maximum crude fat content, 7%) once a day. On the day before the experiment, food was withheld. Morphine sulfate, 1 mg/kg, was administered subcutaneously, and anesthesia was induced 1 hr later with chloralose, 60 mg/kg, and urethane, 600 mg/kg, given intravenously. Thereafter, one-fifth to one-fourth of the anesthetizing dose was injected every 60–90 min to hold an even level of anesthesia. Ventilation with 100% oxygen was assisted through a cuffed endotracheal tube, and the ventilator's stroke volume and rate were regulated to maintain arterial P_{CO_2} between 35 and 45 mm Hg. Hyperinflation to 25 cm H_2O was applied automatically every 5 min to minimize atelectasis. The electrocardiogram was monitored continuously; body temperature was recorded periodically from a thermistor placed

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Abbreviations: TG, triglyceride(s); LVEDP, left ventricular end-diastolic pressure; MAP, mean aortic pressure.

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in the ascending aorta. Catheters were inserted into the left ventricle, the descending aorta, and the inferior vena cava and were kept patent with periodic flushes of 4% sodium citrate. Aortic and left ventricular pressures were detected with Statham P23D strain gauges. Left ventricular end-diastolic pressure was recorded at the highest amplifier gain and was read during end expiration. Zero reference for pressures was set at the midchest level. Cardiac output was estimated from duplicate indocyanine green dilution curves. 1 ml of dye was flushed into the left ventricle, and blood was sampled from the descending aorta through a Gilford densitometer. The blood sample was reinfused after the inscription of each curve. Calibration of the indicator was accomplished through the same catheter system using the animal's blood and three dilutions of dye. The difference between duplicate estimations of cardiac output in these experiments was 5.5 ± 1.2% (mean ± SEM).

To eliminate the adrenergic effects of alcohol and to block any cardiovascular reflexes arising from the anesthetic, the autonomic nervous system was blocked in experiments 11–22. Combined β-adrenergic and postganglionic parasympathetic blockade was produced by an intravenous injection of 0.2 mg/kg of propranolol and 1.0 mg/kg of atropine and maintained by a constant infusion of propranolol, 0.004 mg/kg/min, and atropine, 0.02 mg/kg/min (10).

There were seven periods of observation in each experiment: two control periods at 3–3.5 hr after anesthesia and 30 min after autonomic blockade, and serial observations at 15, 30, 60, 90, and 120 min after starting the infusion of ethanol at rates of 15–30 mg/kg/min. The slowest infusion rate of 15 mg/kg/min was estimated to produce an arterial concentration of ethanol of about 100 mg/100 ml after 30 min and a peak concentration of approximately 200 mg/100 ml by 2 hr (9). The same schedule was followed in experiments using infusions of glucose at 45–48 mg/kg/min (isocaloric to 27 mg/kg/min of ethanol) and 0.85% saline at 2 ml/min. The concentrations of alcohol and carbohydrate were adjusted so that the volume delivered was limited to 240 ml over the 2-hr infusion period. The constant infusion of propranolol and atropine delivered a fluid volume of 1.0–1.5 ml/min, or a total of 180–315 ml for the typical experiment. A specimen of heart tissue was obtained between the two control periods and thereafter periodic samples were taken. An average of 3.6 tissue analyses, including the control sample, were available for the alcohol group, and an average of 3.2 for the glucose–saline group. Arterial blood samples for triglyceride determinations were collected at every period.

Acquisition of tissue specimens

A no. 8F endomyocardial biopsy catheter (Tonokura Medical Industry Co., Ltd., Tokyo, Japan) was used to sample the muscle specimens intravascularly from the endocardium of the right ventricular septum and free wall. The average wet weight of tissue analyzed for triglyceride concentration was 14.4 mg (range 7.2–23.2 mg), which required two to three separate biopsies. With practice, two people could procure three samples in as little as 6 min, including time for maneuvering the catheter from the right external jugular vein into the right ventricle, extracting the sample, and cleaning the catheter before reinserting it. The active steering of the catheter under fluoroscopic monitoring, especially at junctions in the jugular vein, between the superior vena cava and right atrium and at the tricuspid valve was critical to the successful manipulation of the catheter to the right ventricle. The appearance of premature ventricular beats confirmed the correct positioning of the catheter in the right ventricle. At the end of the experiment the animals were killed by exsanguination, and the hearts were examined to assess any trauma from the use of the biopsy catheter and to obtain a right ventricular sample for analysis. The pericardial contents were free of blood except for experiment 13, where the pericardial fluid was tinged with blood. In the earlier experiments, ecchymotic areas (0.5–1.0 cm^2) found in the intima of the superior vena cava and in the endocardium of the posterior right atrium at the atrioventricular groove were due to inexperienced manipulation of the catheter. The result of right ventricular biopsy was usually the presence of endocardial ecchymoses at the biopsy site. Epicardial ecchymoses occurred with biopsies taken from the free wall of the right ventricular endocardium; these were usually confined to areas less than 1.0 cm^2. All of the biopsy specimens taken from one alcohol experiment were analyzed histologically for neutral fat. These samples were fixed in 10% formalin, sectioned during rapid freezing, and stained with oil red O.

Determination of triglyceride concentrations

Each tissue sample was immediately rinsed with cold 0.85% NaCl and dropped into a vial of saline immersed in crushed ice. After the experiment the tissue specimens were blotted gently on filter paper, weighed, and homogenized with saline in 2-ml tissue homogenizers submerged in ice. Blood samples were collected in oxalated tubes that were quickly set in ice. Lipids from both the plasma and the tissue homogenates were extracted with chloroform–methanol 2:1 (v/v) (11). When extracting lipids from the small biopsy specimens, at least two-thirds of the chloroform–lipid phase was dried under nitrogen and spotted on 1 × 3 inch silicic acid plates (Brinkmann, Darmstadt, Germany) that were then developed in a solvent system of ether–acetic acid–hexane 30:1:69. The triglyceride fraction was eluted and the concentration was determined by measuring the glyceride-glycerol moiety (12). Chromatographically pure tripalmitin served as the standard, and its recovery was consistently 97%. Reproducibility of the tissue analysis was established from a total of 36 samples

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TABLE 1. Triglyceride concentrations in right ventricular and left ventricular myocardium

Expt.	Right Ventricle Free Wall	Right Ventricle Septum	Left Ventricle Free Wall	Left Ventricle Septum
	mg of triglyceride/g (wet wt)			
1	0.91 ± 0.03	0.91 ± 0.02	0.91 ± 0.01	0.88 ± 0.01
2	0.95 ± 0.02	0.87 ± 0.02	0.90 ± 0.02	0.91 ± 0.02
3	0.77 ± 0.02	0.74 ± 0.01	0.71 ± 0.01	0.74 ± 0.01

Samples were obtained at necropsy from animals anesthetized with pentobarbital and killed by exsanguination. Concentrations are the means ± SEM of three separate transmural samples determined in triplicate. These same data formed the basis of the reproducibility analysis from which an average SEM of ± 2.1% was calculated.

of myocardium that were extracted singly and measured in triplicate (Table 1). The average standard error of the mean for the triplicate determinations was ±2.1%. Plasma triglyceride concentrations were measured in duplicate, and the difference between the pair was 0.8 ± 0.1 mg/100 ml (mean ± SEM). Despite this agreement between duplicates, we were unable to consistently show a positive arterial–venous difference in concentration across the heart.

The in vivo tissue TG concentrations were analyzed for each animal when sufficient data were available. The regression lines for the relationship between individual concentrations and time were calculated, and the significance of the regressions and the standard errors of the estimates were determined using the *t* test. The hemodynamic results were examined as changes in absolute units from the average of the two control periods. Changes within the ethanol-treated and glucose–saline-treated animals and between the groups were analyzed using the *t* test.

RESULTS

Since the serial biopsies were to be taken solely from the right ventricle, it was necessary to verify that these samples contained triglyceride concentrations representative of both ventricles. From three anesthetized dogs (experiments 1–3) killed by exsanguination, six samples of tissue weighing approximately 1.0 g were taken from each ventricle, base to apex, free wall to septum, for triplicate triglyceride analyses. These data are given in Table 1. Mean concentrations did not differ significantly within the ventricles or between the chambers, and the average SEM was only ±1.8%. From the hearts of seven additional dogs (experiments 4–10), three small samples (weighing approximately 20 mg) from the right ventricular endocardium and a single transmural specimen ninefold larger from the left ventricle were taken at necropsy and compared for TG concentration. The data, summarized in Table 2, show that the concentrations from the two chambers were not significantly different.

TABLE 2. Triglyceride concentrations in right ventricular endocardium and left ventricular myocardium

Expt.	Right Ventricle	Left Ventricle
	mg of triglyceride/g (wet wt)	
4	1.37 ± 0.04	1.48
5	1.10 ± 0.05	1.23
6	1.60 ± 0.14	1.33
7	1.62 ± 0.04	1.46
8	1.74 ± 0.04	1.72
9	1.62 ± 0.04	1.72
10	1.58 ± 0.04	1.72

Samples were taken at necropsy from animals anesthetized with chloralose–urethane and subjected to ethanol infusions of 27–48 mg/kg/min for 2 hr. Concentrations in the right ventricle are the means ± SEM of three endocardial samples that weighed approximately 20 mg each. Concentrations in the left ventricle are solitary measurements from single specimens that averaged 180 mg.

Table 3 tabulates the tissue data from seven dogs infused with ethanol and five dogs administered glucose or saline (experiments 11–22). The mean triglyceride concentration before the infusion in the ethanol group was 0.62 mg/g and was 0.65 mg/g in the combined glucose and saline groups. After the beginning of the ethanol infusions, cardiac TG increased by increments with each successive observation. The regression lines for experiments 11, 15, 16, and 17 were significant at the 5% and 1% levels of confidence. Experiments 12 and 14 ended prematurely with the abrupt onset of hypotension, and the last TG concentrations recorded are those in specimens taken at necropsy. In these two studies, regression lines of all available data gave *P* values of less than 0.10. Thus, with the exception of experiment 11, in which the lowest infusion rate was used, the 15-min samples indicated what was to be a significant trend toward TG accumulation. An analysis of 10 experiments (five from Table 2 and five from Table 3) in which the ethanol infusions continued for 2 hr showed that the concentrations of triglyceride in postmortem tissue correlated with the infusion rate ($r = +0.81$, $P < 0.01$).

In the glucose–saline group, serial TG measurements from tissue taken in vivo and from postmortem tissue did not establish a trend and indicated that general anesthesia and continuous autonomic blockade had negligible effects on the concentration of cardiac TG.

Mean arterial TG concentrations in all animals before and after autonomic blockade were 50.8 and 50.6 mg/100 ml, respectively. In the glucose–saline animals, mean arterial TG after 2 hr was 49.6 mg/100 ml, whereas in the ethanol group, mean arterial TG had risen to 67.2 mg/100 ml.

To assess any consequence of autonomic blockade on hemodynamics, measurements were taken before and after blockade in 19 animals, which included experiments 11–22. Mean values before and 30 min after continuous blockade were, respectively: cardiac output, 114 and 106

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ml/kg/min (NS); left ventricular end-diastolic pressure (LVEDP), 6.5 and 8.6 mm Hg (P = 0.05); and mean aortic pressure (MAP), 130 and 132 mm Hg (NS). After 2 hr in the glucose–saline group, mean changes compared with control were not significant: cardiac output, +6.2%; LVEDP, −0.1 mm Hg; and MAP, −2.0%. Experiment 18 was excluded from the hemodynamic analysis. Of the five experiments, it was the only one that showed a gross change in LVEDP (rising from 7.5 to 31 mm Hg); this was explained by inadvertent administration of an excessive amount of saline.

The results of 2-hr infusions of ethanol on the hemodynamics were compared with control values: cardiac output, −34.0%; LVEDP, +9.9 mm Hg; and MAP, −8.4%. In Fig. 1, the mean differences from controls in actual units are plotted for the ethanol and glucose–saline groups at intervals during the infusions. Significant differences in cardiac output and LVEDP were found between the groups at 15 min and continued for 2 hr. Significant changes from control values within the ethanol group began by 30 min. In the analysis of cardiac function according to the Frank-Starling relationship, a rise in left ventricular end-diastolic pressure is normally associated with an increase in cardiac output. Therefore, the progressive fall in cardiac output despite the rising LVEDP represents impairment of cardiac function (13).

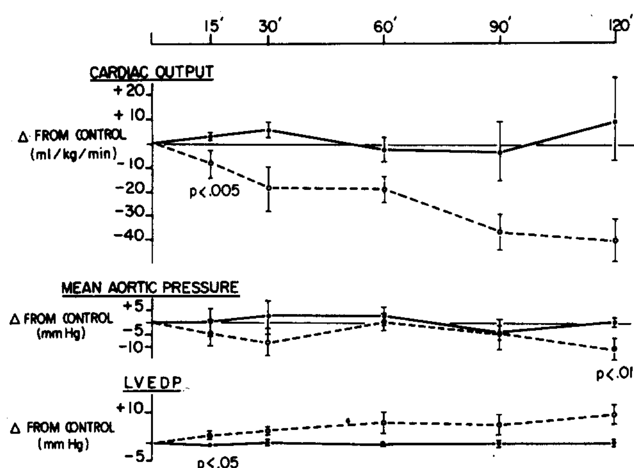

Fig. 1. Mean changes from control in cardiac output, aortic pressure, and left ventricular end-diastolic pressure (LVEDP) were plotted in actual units for the ethanol (open circles) and glucose–saline (closed circles) animals. Limits are 1 SEM. Significant differences in cardiac output and LVEDP were found between the groups after 15 min.

DISCUSSION

Our experience convinced us that procuring multiple endocardial samples was practical and that working with small specimens did not preclude the quantification of TG with a small standard error. Contamination of the tissue measurement from plasma lipid was exceedingly remote. Using Gibson's (14) estimate of blood volume for the dog heart, a plasma TG of 200 mg/100 ml, or three to four times the actual concentrations, would be required for the average tissue increment of 0.07 mg/g for the first 15-min period alone. Moreover, the necropsy specimens were taken from exsanguinated animals and these TG concentrations did not differ from the in vivo concentrations in the saline–glucose group; they were also consistent with the final in vivo measurement in the ethanol group.

The average cardiac TG of 0.64 mg/g taken during the preinfusion control period lies between a low concentration of 0.35 mg/g reported by Regan et al. (9) and a high concentration of 2.8 mg/g reported by Scheuer and Brachfeld (15). These data were obtained from tissues from anesthetized dogs, and the same methods were used. In our own samples, TG concentrations less than 1.6

TABLE 3. Triglyceride concentrations in right ventricular endocardium during infusions of ethanol, glucose, and saline in dogs with the autonomic nervous system blockaded

Expt.	Infusion Rate	Period of Observation (min)						
		Control	15	30	60	90	120	Necropsy
	mg/kg/min	*mg of triglyceride/g (wet wt) of endocardium*						
Alcohol								
11	15	0.66	0.62		0.72		0.84	0.88
12	23	0.71	0.78	0.89		0.98		
13	25	0.56						1.01
14	25	0.51		0.79	1.21			
15	25	0.59	0.69	0.78	0.99	1.08		1.12
16	28	0.68	0.77	0.98	1.24		1.50	1.57
17	30	0.63	0.79	0.98	1.16	1.55		1.62
Glucose								
18	48	0.65			0.61	0.57		0.68
19	46	0.70				0.64		0.64
20	45	0.58			0.63	0.60		
Saline								
21		0.59			0.54		0.57	0.63
22		0.74	0.77		0.70	0.69	0.75	0.67

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mg/g were not detectable histologically with oil red O staining.

We were able to demonstrate that the TG concentration was uniform within and between ventricles and that the small endocardial samples accurately mirrored the overall ventricular concentration. These findings are consistent with the report that the transmural concentrations of TG from the inner to the outer layers of the left ventricle are constant (16). Otherwise, very little quantitative information on the lipid composition of the chambers of the dog heart is available to corroborate or to dispute our data. Barner, Jellinek, and Kaiser (17) reported equivalent concentrations of phospholipid in the two ventricles and the interventricular septum; however, total lipid concentration in the right ventricle was double the concentration in the left ventricle and septum. The difference was presumably made up in part by TG, but it was not reported separately. Gloster and Harris (18) quantitated the lipid composition of the chambers of the dog heart by measuring the lipid in the mitochondrial and microsomal fractions of myocardial homogenates. They found no significant difference in the neutral lipid content between the ventricles.

As for the biopsy procedure itself, we knew that a single biopsy would be harmless (19, 20), but the effect of several biopsies on cardiac performance was unknown. Furthermore, we had not expected to take as many samples as we did, two to three for a single chemical determination. Despite the frequency of biopsy for both groups, cardiac function declined only in the ethanol group and was unaffected in the glucose-saline-treated animals. We are assuming that left ventricular performance parallels right ventricular performance, for even during a short interval these chambers must put out equivalent volumes. Other features in the experimental design, such as 6 hr of general anesthesia and continuous autonomic blockade, might have been expected to depress cardiac function, but these features were also common to both groups. Only alcohol separated the groups hemodynamically; therefore, the impact of the biopsies on cardiac performance as measured was inconsequential.

Autonomic blockade was employed to eliminate the hemodynamic effects of the catecholamine release expected from the ethanol infusion (21). If the dose of alcohol is relatively large, β-adrenergic stimulation releases free fatty acid from peripheral lipid depots through the activation of lipase (22). Therefore, β-adrenergic blockade in these experiments might have modified the effects of alcohol on cardiac metabolism indirectly by altering the concentration of substrates presented to the myocardium. Without data these possibilities are conjectural, but it is noteworthy that the correlation between TG accumulation and infusion rate of alcohol showed a single regression with or without autonomic blockade.

The effects of alcohol on cardiac hemodynamics and lipid content were expected. The new observations were the in vivo measurements of tissue lipid themselves and the rapidity with which deposition began, only 15 min after the start of the infusion. The coincidence between the onset of lipid accumulation and cardiac depression suggests a cause and effect relationship (9). However, it seems unlikely that the physical presence of lipid in quantities too small to see histologically could cause myocardial dysfunction severe enough to be reflected in ventricular filling pressure and cardiac output. On the other hand, the early, simultaneous hemodynamic and metabolic changes are compatible with a direct effect of ethanol on cell or membrane activity, an effect that would influence both contractile and metabolic functions.

This study proved that it is possible to procure from intact animals serial samples of endocardium containing concentrations of TG representative of both ventricles. With existing methods, the examination of these muscle biopsies can be extended to the biochemical and radioisotopic analyses of other classes of lipids. The distribution of other lipids within and between cardiac chambers, especially under different interventions, would have to be confirmed as was accomplished in the present studies. The ultimate application of periodic sampling is the potential for combining dynamic measurements of cardiac lipid simultaneously with the heart's quantitative uptake of fat. Such serial monitoring will make the following in vivo investigations possible: the intermediate metabolism of extracted lipids, the metabolism of endogenous myocardial fat, and the movement between intracardiac and extracardiac pools of lipid. JLR

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