Effects of Excess Free Fatty Acids on Mechanical and Metabolic Function in Normal and Ischemic Myocardium in Swine

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SUMMARY We evaluated the consequences of excess free fatty acids (FFA) on mechanical and metabolic functions in globally perfused working swine hearts. In one group of eight hearts, treatments with heparin and 10% fat emulsion (Intralipid) produced a 3- to 5-fold elevation in serum FFA levels as contrasted with levels in 10 untreated hearts. At control flows, excess FFA caused declines in aortic pressure (−31.4%, P < 0.05), left ventricular systolic pressure (−24.8%, P < 0.05), left ventricular work (−69.8%, P < 0.001), and epicardial motion (−57.8%, P < 0.001), together with an increase in myocardial oxygen consumption (+16.5%, P < 0.05) as compared with pretreatment values. Ischemia in untreated hearts also decreased mean aortic pressure (−46.2%, P < 0.005), left ventricular systolic pressure (−19.5%, P < 0.001), left ventricular max dp/dt (−27.9%, P < 0.001), left ventricular work (−48.1%, P < 0.025), myocardial oxygen consumption (−31.2%, P < 0.001) as compared with preischemic values.

Excess FFA during ischemia resulted in even greater deteriorations in hemodynamic and metabolic functions. Tissue metabolites in the two groups of ischemic hearts were compared with those in six untreated hearts maintained at control flows. Tissue levels (μM/g dry weight) of long-chain acyl CoA and acyl carnitine derivatives during ischemia. Accumulations of these products of fatty acid metabolism may interfere with enzyme functions and membrane transport systems.

FATTY ACIDS (FFA) at high concentrations have been shown to disrupt cellular processes in a variety of tissues. Several mechanisms have been implicated to explain these derangements, including a nonspecific detergent action on biomembranes, inhibition of enzymes, uncoupling of oxidative phosphorylation in mitochondria, and stimulation of mitochondrial ATPase. In ischemic myocardium, either in the clinical or experimental setting the aforementioned actions are thought to result in the genesis of serious dysrhythmias. Threshold levels of FFA at which arrhythmias occur vary from 1200 μEq/liter in man to 3000 μEq/liter in dogs. Fatty acids also may impair mechanical function in ischemic hearts, although the mechanisms and threshold values have not been defined completely. One cause may be the recently demonstrated inhibitory effects of long-chain acyl CoA esters on adenine nucleotide translocation across mitochondrial membranes which impair ATP egress from the matrix to the cytosol. A second cause may be inhibition of membrane Na+, K+-stimulated ATPase by long-chain acyl CoA and long-chain acyl carnitine which could lead to disruption of critical transport processes. In the past, studies of the toxicity of fatty acids in ischemic hearts primarily have involved coronary ligation and infusions of large amounts of lipids which produced extremely high levels of extracellular FFA (2350 to 10,000 μEq/liter) in experimental animals. These extreme conditions may mask the early effects of moderately high FFA and mild ischemia on mechanical performance and explain certain of the disparities that exist between experimental and clinical data.

The purpose of our studies, therefore, was to document the effects of moderate increases in FFA in a setting of early myocardial ischemia. Particular emphasis was directed toward determining the interrelationships between intracellular accumulation of acyl CoA and acyl carnitine derivatives and mechanical and metabolic dysfunction.

Methods

Twenty-four swine of either sex, weighing 33.6-55.0 kg (average 43.9 kg) were studied follow-
Preparation and Instrumentation

We produced global myocardial ischemia in an intact, working heart preparation in open-chested swine. Following bilateral thoracotomy with transthorotomy and treatment with heparin (3 mg/kg, iv), two separate extracorporeal perfusion circuits were constructed connecting both cannulated femoral arteries to the main left and right coronary arteries. The main left coronary artery was perfused via a Gregg cannula inserted retrograde through the left subclavian artery, and the right coronary artery was perfused by a cannula positioned near its origin. Flow was determined in both systems by adjusting the respective mean perfusion pressures to slightly above average aortic pressure to compensate for perfusion line resistances. Flows to each artery were controlled by separate low-flow Sarnes perfusion pumps, model 6050. To sample metabolites and arteriovenous differences of oxygen across the myocardium, a venous cannula also was passed into the coronary sinus from a lateral insertion in the great cardiac vein. The hemiazygos vein, which drains directly into the coronary sinus, had been ligated previously.

Additional cannulas were placed in the left ventricle and distal aorta to measure pressures. A high-fidelity, manometer-tipped pressure device (Statham model P 866) was advanced retrogradely from an internal carotid artery to the left ventricle to determine left ventricular pressure. A Teflon catheter, 0.10 inch i.d., connected proximally to a P23Db pressure transducer, was also inserted retrogradely into the descending aorta via an internal mammary artery. Signals from both transducers, with the electrocardiogram, were displayed on an eight-channel Mark 200 Brush recorder. Signals from the high-fidelity, pressure-tipped catheter were differentiated by a direct-coupled, solid state, constant input-impedance differentiator which had a zero phase shift from zero to 90 Hz.

A specially designed, high frequency displacement transducer, anchored to the heart by 4-mm plunge probes and sutures, was positioned on the anterior epicardial surface near the apex to measure motion. This device operated on the principles of a Wheatstone bridge in which changes in epicardial displacement were sensed as variations in electrical resistance. The gauge consisted of two separate and movable components, one of which was a stiff, steel-alloy wire attached at a 90° angle to one of the plunge anchor probes. This wire was inserted into a short piece of saline-filled polyethylene tubing attached to the second component of the gauge. The polyethylene tubing of this component was in turn joined to a longer piece of Silastic tubing, through the center of which was passed a second thin flexible wire which attached firmly to the polyethylene tubing. These two wires, one embedded into the myocardium, the other passing through to external instrumentation (excitation source and signal processing), thus interrelated through a saline gap, the resistance of which varied with its length. Preliminary bench studies using these transducers demonstrated a flat frequency response in excess of 80 Hz with a response time to 95% steady state displacement of 3 msec.

Data Collection

Estimates of global left ventricular pump performance were made from measurements of heart rate, left ventricular (LVP) and mean aortic pressures, and the maximum rate of left ventricular isovolumetric pressure development (LV max dp/dt) at normal and ischemic coronary flows. These were correlated with regional measurements of epicardial displacement in lengths (L) and an integrated index of work obtained throughout a reconstructed cardiac cycle (work = \( \int \text{LVP} \cdot \text{dt} \)).

Data were collected on-line at 10-minute intervals during the experiments and reduced off-line using a Digital Equipment Corporation PDP 11/10 computer. From each sampling time, 240 data points per cardiac cycle were obtained for 10 consecutive heart beats at held expiration to define an average representative beat. Any cycle which deviated by more than two standard deviations from previous beats was excluded from the averaging routine.

General metabolic function was evaluated from the rate of myocardial oxygen consumption. Oxygen-carrying capacity for hemoglobin was considered uniform in each swine. Oxygen saturation (O₂ sat) was measured with an Instrumentation Laboratory Co-Oximeter, model 182. Myocardial oxygen consumption (MVO₂) was calculated from coronary flow, coronary perfusate hemoglobin (Hb) concentration, and hemoglobin oxygen saturation according to the expression:

\[
\text{MVO}_2 = 1.39 \times \text{coronary flow rate (ml/hr)} \times \text{Hb conc (g/100 ml)} \times \text{arterial-venous O}_2 \text{sat (Å%/22.4 (ml/mmol))} \times \text{dry wt of heart (g)}
\]

Serum also was obtained at 10-minute intervals to determine the total fatty acid content using the colorimetric procedure of Duncombe. Fatty acid levels were expressed both in absolute terms (μM) and per gram of serum protein as measured by the Lowry procedure. On completion of the perfusion trials, transmural sections of left ventricular myocardium near the apex were removed immediately and frozen between blocks of aluminum cooled in liquid nitrogen.

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These tissue samples were analyzed for acid-soluble and long-chain acyl CoA and acid-soluble and long-chain acyl carnitine. The frozen tissue was powdered and extracted in a cold mortar containing cold 6.0% (wt/vol) perchloric acid (PCA). The tissue-PCA mixture was spun for 10 minutes at 12,000 rpm in a Beckman J-21B centrifuge. The supernatant extract was retained for analysis of acid-soluble intermediates while the tissue pellet, after further rinsings with 0.6% PCA to remove any remaining traces of acid-soluble compounds, was used to estimate the long-chain intermediates. For CoA measurements, the supernatant extract was mixed with 10 mM dithiothreitol (DTT) and hydrolyzed at pH 11.0 and 37°C for 1 hour to be sure all acid-soluble CoA was in the form of free CoA. Similarly, for long-chain acyl CoA, the rinsed tissue pellet was resuspended in 10 mM DTT and hydrolyzed at pH 11.5 and 55°C for 10 minutes and analyzed for free CoA. Free CoA was measured enzymatically on a fluorometer.18

Similar procedures were used to measure acid-soluble and long-chain acyl carnitine from the supernatant extract and tissue pellet, respectively. The supernatant extract was hydrolyzed at pH 12.5 and 37°C for 1 hour to ensure that all carnitine was present as free carnitine. The protein pellet was resuspended in water and hydrolyzed at pH 12.5 and 70°C for 1 hour to convert long-chain acyl carnitine to the free form. Free carnitine was then measured in each case by a radioisotopic technique using acetyl-CoA [1-14C].19

Additional tissue was processed to estimate the myocardial content of potassium,20 ATP, and creatine phosphate.21

**Experimental Protocol**

The general plan of these studies was to evaluate the effects of excess fatty acids on left ventricular function during mild global ischemia and to correlate these with tissue levels of key intermediates of fatty acid metabolism. Three groups of perfused hearts were compared. In group 1 (10 pigs), coronary flow was maintained at control levels for 50 minutes, after which flow was gradually reduced over 10 minutes to mildly ischemic levels (35.7% of control flows) for an additional 30 minutes. Data on mechanical performance and metabolism were sampled initially and at 10-minute intervals throughout the control and ischemic perfusion periods (0–60 minute perfusion times in the figures). All pigs were treated with 50–100 mg lidocaine, iv, to reduce the occurrence of ventricular dysrhythmias. Hearts in group 2 (eight pigs) also were perfused at normal flows for 50 minutes before inducing mild ischemia but, in this group, heparin (0.75–1.0 mg/kg) and Intralipid (150 ml delivered at 5 ml/min) were infused during the first 30 minutes (~30 to 0 minute perfusion times in the figures). Intralipid (10%) is a fatty emulsion made by Cutter Laboratories and consists of 10% soybean oil (with a fatty acid composition of 54% linoleic, 26% oleic, 9% palmitic, and 8% linolenic acids), 2.25% glycerin, and 1.2% egg yolk phospholipids. Additional 50-ml doses of Intralipid were given at 0, 20, and 40 minutes of perfusion in an attempt to keep the augmented serum fatty acid levels constant. At the end of the 30-minute ischemic period, or with the onset of ventricular fibrillation which occurred in two fat-treated ischemic hearts during ischemic failure, transmural tissue samples were immediately taken for later analyses. A third group of control hearts (six pigs) was perfused with normal coronary flow and serum fatty acids for 90 minutes, after which tissue samples were obtained. The data were analyzed using paired and unpaired Student’s t-tests with statistical significance defined for probability values less than 5%. The data in the Results section are listed as the mean ± SEM value.

**Results**

**Effects of Flow Reduction of Myocardial Function**

In the 10 pigs of group 1 (body weights 42.1 ± 1.7 kg; heart weights 30.0 ± 1.3 g dry) serum FFA concentrations averaged 0.34 ± 0.02 μM/ml or 5.35 ± 0.32 μM/g protein (Fig. 1). During the control period when coronary flow was maintained at 167.1 ± 10.0 ml/min (between −30 and +20 minutes of perfusion in the figures), no significant changes (by paired Student’s t-test comparisons) were noted in global hemodynamic function (Fig. 2), myocardial oxygen consumption, and/or coronary perfusion pressures (Fig. 3). Regional mechanical function as estimated by epicardial motion and an integrated index of left ventricular work (both normalized to initial values at −30 minutes) were either stable (motion) or improved (work) from −30 to +20 minutes of perfusion (Fig. 4); work increased significantly (P < 0.01).

Following control measurements, coronary flow was reduced over 10 minutes to 107.4 ± 6.0 ml/min (Fig. 1). A gradual rather than an acute reduction in flow was employed to avoid the increased risks for developing malignant ventricular dysrhythmias that previously were shown to occur with precipitous falls in coronary flow in this model.22 These adjustments in flow caused a decline in perfusion pressures in each perfusion circuit to approximately 60 mm Hg, a level previously demonstrated to effect mild ischemic changes.24 There were progressive decreases in almost all indices of mechanical performance (Figs. 2–4). By the end of the ischemic period, mean aortic pressure had fallen by 46.2% (P < 0.005); left ventricular systolic pressure had decreased by 19.5% (P < 0.001); left ventricular max dp/dt, by 27.9% (P < 0.001); and left ventricular work, by 49.1% (P < 0.025). Heart rate and epicardial motion were statistically unchanged. Commensurate with the decline in mechanical function and coronary flow, myocardial oxygen consumption (Fig. 3) decreased by 21.2%, P < 0.001.
EXCESS FFA IN NORMAL AND ISCHEMIC MYOCARDIUM/Liedte et al.

This excess in FFA effected a significant decrease (by paired Student’s t-test comparisons) in mechanical performance at control flows (0–20 minutes of perfusion in Figures 2 and 4). In comparison to initial values, decreases were noted in aortic pressure (–31.4%, P < 0.05), left ventricular systolic pressure (–24.8%, P < 0.05), work (–69.8% P < 0.001) and epicardial motion, (–57.8%, P < 0.001). Heart rate increased by 14.7% (P < 0.05), as did myocardial oxygen consumption, (+16.5%, P < 0.05) (Fig. 3).

These changes were confirmed by contrasting (by Student’s t-tests) the mechanical and metabolic data between group 2 and group 1 pigs over −30 to +20 minutes of perfusion. At comparable coronary flows, a progressive deterioration in mechanical function was again noted. By the end of 20 minutes of control perfusion, the differences between groups were significant: mean aortic pressure (P < 0.05), left ventricular systolic pressure (P < 0.025), max dp/dt (P < 0.05), epicardial motion (P < 0.001), left ventricular work (P < 0.001), and myocardial oxygen consumption (P < 0.025). Heart rate was unchanged.

Effects of Excess Serum Fatty Acids during Ischemia

Data from group 1 and 2 pigs obtained between +20 and +60 minutes of perfusion were compared by unpaired Student’s t-tests. Two pigs in the FFA-supplemented group died during this period, resulting in a mean survival time of +53.9 ± 4.1 minutes of perfusion. Mechanical and metabolic data in the excess FFA group were averaged for this final time and included the last recorded information for all pigs. These were compared statistically with the low FFA data at both 50 and 60 minutes of perfusion. At least a 3-fold difference in serum fatty acids was maintained between groups during the ischemic perfusion. The increase in FFA in group 2 hearts resulted in a progressive and significant deterioration in hemodynamic function as compared with group 1 hearts at comparable times and levels of ischemic perfusion. At the termination of the studies, mean aortic pressure in FFA-supplemented pigs was 39.3% below that of low FFA pigs (P < 0.05); left ventricular pressure was reduced (–29.0%, P < 0.025) as were max dp/dt (–60.7, P < 0.01), epicardial motion (–65.2%, P < 0.05), and left ventricular work (–63.1%, P < 0.05). Myocardial oxygen consumption was reduced by 18.4% (P < 0.05). Although perfusion pressures were statistically comparable in the right coronary perfusion circuit (average, 61.6 mm Hg), pressures in the left coronary perfusion circuit were significantly less in group 2 (–18.7%, P < 0.005) at final measurement. Even greater statistical differences were observed when the terminal data obtained in group 2 were compared with those at +50 minutes of perfusion in group 1 pigs (Figs. 1–4).

Effects of Excess Serum Fatty Acids at Control Coronary Flows

In the eight pigs of group 2 (body weights 46.2 ± 2.4 kg; heart weights 29.8 ± 1.8 g dry), coronary flow averaged 183.6 ± 14.2 ml/min during the control perfusion period (not statistically different from control flows in group 1 pigs). Infusions of Intralipid with heparin resulted in a 5-fold increase in serum fatty acids at the end of the pretreatment phase (0 minutes perfusion time in the figures), after which FFA fell somewhat despite added supplements.
Tissue Levels of Fatty Acyl Intermediates and Other Metabolites at Control and Ischemic Flows

Emphasis was placed on relating the above changes in mechanical function and metabolism to tissue stores of fatty acid metabolites. The data obtained from the two groups of ischemic hearts were compared with a third group of six hearts maintained at control flows (169.4 ± 12.4 ml/min) throughout the perfusion periods. Hemodynamic and metabolic variables were measured every 20 minutes in the normal flow group and included: heart rate (121.0 ± 2.5 beats/min), mean aortic pressure (82.7 ± 3.7 mm Hg), left ventricular peak systolic pressure (97.1 ± 2.4 mm Hg), left ventricular end-diastolic pressure (6.4 ± .7 mm Hg), and myocardial oxygen consumption (1.02 ± .07 mM/hr per g). There was no deterioration in function over the course of perfusion in this group (by paired Student's t-test comparisons), nor were there differences at comparable times between this group and the preischemic data in the low FFA group (by unpaired Student's t-test comparisons). Tissue stores of fatty acyl CoA and carnitine derivatives are shown in Figure 5. Ischemia per se effected a near 2-fold increase in long-chain acyl CoA (P < 0.001 compared with control data) and an 18-fold increase in long-chain acyl carnitine (P < 0.001). There were associated decreases in acid-soluble (free and acetyl) fractions of both CoA and carnitine. Ischemia together with excess FFA effected an increase of 2.5 times in long-chain acyl CoA over control levels (P < 0.001) and a 33-fold increase in long-chain acyl carnitine. Total carnitine stores were significantly decreased in the ischemic-high FFA hearts which may reflect a generalized leakage of cytosolic contents.

In the same three groups of hearts, tissue also was obtained for measurements of myocardial contents of potassium, ATP, and creatine phosphate. The potassium data, expressed in mEq/g wet weight were: control hearts, 0.073 ± 0.004; ischemic hearts, 0.071 ± 0.006; and fat loaded ischemic hearts, 0.073 ± 0.009. Control data were similar to that previously reported for swine hearts,24 and there were no statistical differences between or among groups. The high-energy phosphate data, expressed in µM/g dry weight, were: control hearts, ATP 21.5 ± 1.2, creatine phosphate 38.5 ± 2.8; ischemic hearts, ATP 9.8 ± 0.1, creatine phosphate 23.5 ± 2.4; and fat-loaded ischemic hearts, ATP 12.0 ± 1.0, creatine phosphate 17.2 ± 2.0. Both ATP and
**Discussion**

This study was designed to evaluate the early effects of excess FFA on cardiac performance. Serum fatty acid levels were increased only moderately to evaluate the early threshold changes in function and metabolism. Both at control and mildly ischemic coronary flows, FFA significantly depressed regional and global mechanical function. These changes were associated with a progressive build-up of long-chain fatty acyl CoA and carnitine derivatives in ischemic hearts. Before considering possible interpretations of these data, a critique of the methods and protocol is presented first.

Fat-loading in group 2 pigs was accomplished by infusions of Intralipid and heparin. The fat emulsion contains 97% fatty acids and has been used extensively in past studies to induce fatty acid excess. Neither the small portion of emulsifying agents (egg yolk phospholipid and glycerin) in the fat mixture nor the heparin has been implicated previously to depress cardiac function, and we conclude that the major effects described in this study resulted from the FFA.

Fatty acids have been shown both in clinical and experimental studies to induce ventricular dysrhythmias. In our study, all pigs were pretreated with lidocaine to avoid such dysrhythmias, since major emphasis was directed toward influencing mechanical and metabolic functions. Only two instances of ventricular fibrillation were observed in the FFA-treated group, and these were agonal occurrences following the progression of advanced mechanical failure.

In this study, left ventricular end-diastolic pressure did not rise appreciably during ischemic perfusions. Of concern in the FFA-treated group was whether such a trend might be masked by an alteration in preload resulting from the fat emulsion particles plugging a large portion of the pulmonary microvasculature. Against this was the size of the particles in the fat emulsion (approximately 0.5 μm), much smaller than that required to obstruct most capillaries, and the absence of any significant increase in end-diastolic pressures in untreated group 1 hearts. This suggested rather that the ischemic exposure in both groups was not of sufficient intensity or duration to produce changes in end-diastolic pressure.

With regard to metabolism, it was elected not to quantify the rates of oxidation of the several fatty acids by radioisotopic labeling because of the difficulty in identifying the specific activities of the fatty acid pool. However, it has been previously demon-
stratized in an analogue model in swine hearts that the rates of fatty acid oxidation decrease during ischemic perfusion.22

Previous data have been published for tissue levels of acyl CoA and carnitine derivatives in control and ischemic rat hearts perfused with 11 mM glucose and 1.0 mM palmitate.26,27 Our data for swine hearts compared well with these data for long-chain acyl CoA but were lower for long-chain acyl carnitine. To check against possible methodological error and specifically to exclude potential loss of long-chain acyl carnitine during the process of pellet rinsing, repeat analyses were made. First, tissue was randomly selected from several of the control and ischemic hearts (n = 8) and processed to determine total carnitine.18,19 These were compared with the values derived from summing the long-chain and acid-soluble carnitine fractions as shown in Figure 5. The results were: 3865.8 ± 244.0 μM/g dry (derived arithmetically) vs. 3559.3 ± 329.7 μM/g dry (measured directly). These were not different statistically. Next, the specific effects of rinsing per se were tested. Tissues (n = 5) from fat-loaded ischemic hearts (in which losses of total tissue carnitine were greatest) were processed identically in duplicate runs with the single exception that in one series the tissue pellets were rinsed and in the other they were not. In the latter trials, the acid-soluble carnitine in the pellet was calculated and subtracted to get the long-chain value. The results were for the rinsed trials: long-chain carnitine 602.5 ± 136.0 μM/g dry; and acid-soluble carnitine 3147.8 ± 317.4 μM/g dry. For the non-rinsed series these were: long-chain carnitine 630.3 ± 189.7 μM/g dry; and acid-soluble carnitine 3194.8 ± 278.4 μM/g dry. The data from these studies compared closely with those of the original results and indicated that rinsing did not remove significant portions of long-chain acyl carnitine. As a last check we prepared additional rat hearts (three control, four ischemic) similar to those previously described.26,27 Coronary flow in ischemic hearts was reduced by 57 ± 3%. Data (averaged for rinsed and unrinsed trials) from control hearts included: long-chain carnitine, 411.2 ± 132.8 μM/g dry and acid-soluble carnitine, 5544.7 ± 386.3 μM/g dry; and from ischemic hearts: long-chain carnitine, 1961.8 ± 79.3 μM/g dry; and acid-soluble carnitine, 3096.1 ± 237.4 μM/g dry. Given the smaller number of hearts assayed and the variation between batch analyses, the data were comparable with those previously reported. However, a persistent difference in absolute levels of long-chain carnitine remained between rat and swine hearts. Possible explanations include differences in either species, method of perfusion and oxygen availability of the coronary perfusate, levels and duration of ischemia, and/or levels and types of FFA loading.

Finally, we elected to include a measure of regional motion in this model of global perfusion. We recognize that this information is region dependent and were careful to attach the displacement transducers at a similar location on the anterior surface of the heart near the apex. Still, in globally perfused hearts, there need not be complete uniformity of contractile motion in response to adjustments in coronary flow. Such characteristics may explain in part the wide range of changes in motion and work noted during both control and ischemic flows, particularly in untreated hearts (Fig. 4). We did observe a difference in the patterns of contraction between this model and that previously reported for regional ischemia.28 Diastolic shortening, of major consequence in regional ischemia, was not nearly as pronounced during global ischemia. Aneurysmal systolic bulging, a common feature of regional underperfusion, was seen in only three globally ischemic hearts and only at the terminal sampling times.
Previous studies have established that FFA, if supplied in high enough doses, can disrupt cardiac performance. Mechanisms to explain these actions are not as yet completely understood. It has been suggested that dysrhythmias result from an interference by fats on glycolytically derived ATP in the cytoplasm or from biotoxic detergent effects on membranes enhanced by high molar ratios of FFA-albumin. This latter “unbinding” of FFA is influenced by the chain length and/or configuration of the FFA involved and by the pH of the perfusate. In the present study, pH was determined in coronary arterial and venous blood at control and ischemic flows. At the level of FFA loading and ischemia studied in group 2 hearts, the buffering capacity of the blood was sufficient to prevent acidosis from developing. This would argue against “unbinding” of FFA from albumin as being of major importance. Furthermore, in group 2 pigs, serum concentrations of FFA averaged 25.7 μM/g protein. This yielded a FFA-albumin molar ratio of 4.5:1 (assuming a molecular weight for albumin of 65,000) which is well beneath the thresholds for the development of dysrhythmias as previously reported.

Previous studies have shown that FFA interferes with mitochondrial phosphorylation. In effect, FFA reduces the efficiency of mitochondrial respiration through an uncoupling of electron transport such that myocardial oxygen consumption is increased. This action was seen in the present study at control flows. Accompanying this was a decline in hemodynamic performance. By comparing an arbitrarily defined mechanical-metabolic efficiency ratio of left ventricular work-myocardial oxygen consumption, the presence of excess FFA reduced this ratio by 56% at the end of control
perfusions, and this was greater than the decline noted with ischemia in low-fat hearts (−21%). Excess FFA caused a further terminal decline in oxygen consumption during ischemia and a 23% reduction in the efficiency ratio as compared with low FFA hearts at the end of the ischemic phase. Fatty acids in sufficient quantities have been reported to impair mechanical performance. Our data showed a striking decline in both regional and global mechanical functions at control and ischemic flows. These changes occurred at lower FFA concentrations than previously reported and suggested that the threshold values of FFA for mechanical dysfunction (molar ratio of 4.5:1, FFA-albumin), previously not known, are below those described for their dysrhythmogenic effects (7:1, FFA-albumin molar ratio as reported by Willebrands et al.).

Attention recently has focused on the effects of FFA on enzymes and transport functions in membranes as a cause of inhibition of cardiac performance. Ahmed and Thomas originally found that free fatty acids inhibit brain microsomal Na+, K+-ATPase. Both carnitine and acyl CoA esters of long-chain fatty acids now have been reported to act similarly on cardiac membranes. Long-chain acyl CoA (but not acyl-carnitine) also has been observed to inhibit mitochondrial translocation of adenine nucleotides. With this in mind, the purpose of the present study was to measure the intraacellular accumulation of these compounds both as a function of ischemia and fatty acid concentration. Following more marked ischemic exposures in rat hearts (>60% reductions in coronary flow), Neely et al. documented 2- to 3-fold increases in tissue concentrations of long-chain acyl CoA and acylcarnitine, probably due to limited metabolism by β-oxidation in mitochondria. In our swine hearts, similar increases in fatty acyl CoA groups were noted at less severe levels of ischemia but were accompanied by far greater percentage increases in acyl carnitine. With the addition of excess fatty acids in ischemic hearts there were even greater and near-linear increases in these products, particularly for long-chain acyl carnitine (33-fold increase over control concentrations). In an effort to further define possible membrane dysfunctions as a result of these accumulations, tissue levels of potassium, ATP, and creatine phosphate were determined. Ischemia resulted in significant decreases in high-energy phosphates but not in potassium. Fat-loaded hearts had slightly more ATP, slightly less creatine phosphate, and similar levels of potassium, compared with untreated hearts. However, the absolute differences in high-energy phosphates were small and, without knowing their exact distributions in cytosol and mitochondria, it is premature to conclude possible alterations in adenine nucleotide translocase activity. Present methods for intact tissue probably are too inexact to make such a determination.

However, it has been estimated that some 90-95% of carnitine and its acyl derivatives are located in the cytosol of cardiac cells. The increase in acyl carnitine observed in the present study would provide concentrations in the cytosol sufficient to inhibit Na+, K+-ATPase. Secondary effects on membrane translocation of adenosine nucleotides could also result from the conversion of excess acyl carnitine to long-chain acyl CoA. Thus, these data suggest that one effect of elevated serum FFA on metabolic and mechanical functions in ischemic hearts may act through cellular accumulations of inhibitory intermediates which compromise the integrity and performance of cardiac membranes.

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