Effect of Free Fatty Acids on Myocardial Function and Metabolism in the Ischemic Dog Heart

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ABSTRACT Since elevation of plasma concentrations of free fatty acids (FFA) increases myocardial oxygen consumption without influencing mechanical performance in normal hearts, it was the purpose of this study to determine whether FFA would modify mechanical performance at limited oxygen supply. Left coronary blood flow was reduced by gradual clamping of a shunt from the left carotid artery until moderate ventricular dilatation supervened. Left ventricular systolic pressure (LVSP), its maximal rate of rise (dP/dt) and stroke volume (SV) were unchanged or slightly reduced. The ischemia resulted in a decrease in myocardial oxygen consumption ($M\dot{V}O_2$) from 9.7±1.1 ml/min to 7.9±0.8 ml/min, and myocardial lactate uptake was reduced or reversed to excretion. Increasing the plasma concentrations of FFA from 359±47 μEq/l to 3688±520 μEq/l by intravenous infusion of a triglyceride emulsion and heparin resulted in further ventricular dilatation, accompanied by increased excretion of lactate. The ventricular decompensation and enhancement of anaerobic myocardial metabolism associated with increased uptake of FFA was not related to changes in coronary flow, $M\dot{V}O_2$, or LVSP. dP/dt and SV were virtually unchanged. Intravenous infusion of glucose/insulin, which lowered plasma concentrations of FFA, reversed ventricular dilatation and lactate excretion.

The data support the hypothesis that high concentrations of FFA play a significant role in increasing myocardial oxygen requirement and thereby promote depression of contractility of the hypoxic heart in experimental animals.

INTRODUCTION

Recent findings have suggested that factors influencing the balance between myocardial oxygen supply and demand can substantially alter the extent of myocardial ischemic injury following acute coronary occlusion (1). Thus, in conditions of reduced myocardial perfusion and oxygen supply it is conceivable that factors stimulating myocardial metabolism might lead to further impairment in myocardial contractility, with attendant "pump failure."

Raised arterial concentration—and consequently myocardial uptake—of free fatty acids (FFA)[1] has been shown to increase myocardial oxygen consumption in the intact heart without altering myocardial performance (2–5). Consequently, the effect of high concentrations of FFA is to increase the ratio between the oxygen utilized by the heart and the work performed. The calorigenic effect of FFA occurs both during i.v. administration (2, 5, 6) and during lipolysis induced by catecholamines from endogenous lipid stores (3, 4, 6).

It is well documented in man that arterial levels of FFA increase during acute myocardial infarction (7). Recent reports suggest a correlation between the concentration of plasma FFA and mortality in patients with ischemic heart disease (8), and provide experimental evidence that FFA have an arrhythmogenic effect on infarcted hearts (9). Studies on isolated heart preparations have recently demonstrated a depressant effect of FFA on muscular mechanics (10, 11). It is therefore possible that the increased oxygen requirement induced by FFA is a contributory factor to myocardial cell injury after coronary occlusion, thus being of importance in the development of myocardial "pump failure."

This hypothesis was tested in the intact dog heart by producing graded and controlled myocardial ischemia. The temporal relation between myocardial oxygen consumption and ventricular dilatation—as evidenced by in-

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[1] *Abbreviations used in this paper:* LVP, LVSP, LVDP, LVEDP, left ventricular, systolic, diastolic, and end-diastolic pressure, respectively; dP/dt, maximal rate of LVP rise; MCL, myocardial chord length; EDMCL, end-diastolic MCL; HR, heart rate; SV, stroke volume; $M\dot{V}O_2$, myocardial oxygen consumption; FFA, free fatty acids.

traventricular distance gauges (12)—was evaluated before and during elevation of arterial FFA by intravenously infusing a triglyceride emulsion and activating the plasma lipolytic system with heparin (13).

METHODS

Animal preparation. 23 mongrel dogs, weighing 15–25 kg, were anesthetized with sodium pentobarbital (25 mg/kg) given i.v. Ventilation was maintained with the use of a Cyclator Mk 2 (The British Oxygen Company, Ltd., London, England) positive pressure respirator. A left thoracotomy was performed through the fifth intercostal space. The left ventricular descending and circumflex arteries were cannulated close to the bifurcation of the left main coronary artery, and perfused from the left carotid artery, after adequate heparinization (sodium heparin 5 mg/kg).

Hemodynamic determinations. Aortic and coronary flow rates were determined by an electromagnetic square-wave flowmeter (Nycotron, Oslo, Norway); aortic flow with a probe on the ascending aorta, and coronary flow with an extracorporeal probe in the shunt to the coronary arteries. Left ventricular pressure (LVP) (0–200 mm Hg) and left ventricular diastolic pressure (LVDP) (0–40 mm Hg) were recorded through a cannula inserted into the apex of the ventricle. The first derivative of LVP (dP/dt) was obtained continuously with a differentiating unit from the output signal of the pressure channel. LVP and pressure in the shunt were measured by a Statham transducer (P 23 Gb; Statham Instruments, Inc., Oxnard, Calif.). Instantaneous changes in left ventricular dimensions were monitored with pairs of ultrasound distance gauges, as previously described (12). Briefly, one or two pairs of piezoelectric ceramics of lead zirconate titanate (0.5·1·3 mm) were sewn into the left ventricular wall, 4–5 mm below the surface. The distance between them—the myocardial chord length (MCL)—was measured by the transmission time for ultrasound pulses (1000/sec) from one element serving as transmitter of the pulses, to the other serving as receiver. Distance between elements was 8–14 mm. The signal was sensitive to changes in distance as small as 0.01 mm. Flow, pressures, and MCL were recorded on a Hewlett-Packard multichannel recorder (Hewlett-Packard Co., Palo Alto, Calif.). Blood was sampled from the cannula in the left ventricle and from a catheter inserted into the coronary sinus. Portions of arterial and coronary sinus blood were sampled simultaneously and analyzed for oxygen content, lactate, and FFA. In vitro lipolysis was avoided by placing blood immediately into tubes chilled in ice water and centrifuged for 10 min at 2°C (9).

Metabolic determinations. Oxygen saturation was determined spectrophotometrically by the micromethod of Aukland (14), and hemoglobin was measured as cyanmethemoglobin. Oxygen content was calculated using 1.34 ml O_2/g hemoglobin. Myocardial oxygen consumption ($M\dot{V}O_2$) was calculated from myocardial blood flow and the coronary arteriovenous difference of oxygen. Lactate was measured in duplicate, according to the method of Hohorst (15). Myocardial lactate uptake/excretion was expressed as the product of coronary arteriovenous lactate difference and coronary blood flow in micromoles per minute. Measurements of plasma FFA were made in duplicate, according to the method of Dole, as modified by Trout, Estes, and Friedberg (16, 17). Myocardial uptake of FFA was calculated as the product of coronary arteriovenous difference and coronary plasma flow, and expressed in microequivalents per minute.

Probability values for differences between paired data were obtained with Student's *t* test.

Experimental procedure. Ventricular dimensions, LVP, and stroke volume (SV) were similar before and after the shunt to the coronary arteries had been established. Pressure/flow curves obtained by graded constriction of the shunt revealed intact autoregulation in all experiments. By these criteria the shunt did not interfere with the myocardial function or hemodynamics.

The animals were divided into three groups. In the first group (four dogs), experiments were performed to confirm the effect of FFA on $M\dot{V}O_2$ (2–5) during free coronary flow in the present preparation. After control observations, infusion with a triglyceride emulsion (Intralipid, Vitrum,[2] Stockholm, Sweden, 4 ml/min) was begun. A supplementary dose of heparin (3 mg/kg) was given to secure maximum lipolytic effect. After 10 min of infusion, hemodynamic and biochemical parameters were redetermined.

In the second group (nine dogs), the relationship between metabolic parameters and the degree of ventricular dilatation was studied during graded reduction in myocardial oxygen supply. Coronary blood flow was reduced by means of an adjustable clamp in steps of 10 ml/min until significant myocardial ischemia occurred. This state was signaled by the onset of ventricular dilatation. Measurements were made at two steps of ischemia during steady hemodynamic state and after stable myocardial dimensions were obtained.

In the third group (10 dogs), the effects of increased arterial concentration of FFA during ischemia were studied. After control measurements, coronary perfusion pressure was reduced until ventricular dimensions were significantly increased, but not to an extent that would markedly depress systolic left ventricular pressure (LVSP) and SV. After an equilibration period of not less than 5 min in steady state with constant ventricular dilatation, all determinations were repeated. An i.v. infusion of homologous blood (4 ml/min)—given throughout the preceding period in order to avoid changes in preload—was thereafter replaced by infusion of a triglyceride emulsion (Intralipid, 4 ml/min). A supplementary dose of heparin (3 mg/kg) was given in order to secure maximum lipolytic effect. When a new steady hemodynamic state was established—usually within 5 min, and never exceeding 10 min—hemodynamic and biochemical parameters were redetermined.

In four dogs coronary constriction was maintained and administration of glucose 5.5% (4 ml/min, i.v.) and insulin (40 U/500 ml) began, replacing the infusion of lipid. 15 min later hemodynamic and metabolic parameters were redetermined.

RESULTS

Effects of FFA on myocardial hemodynamics and metabolism during free coronary flow (Group 1). Infusion of Intralipid/heparin during unrestricted coronary flow caused no hemodynamic changes (Table I). Myocardial

[2] 100 ml contains: fractionated soya bean oil, 10 g; fractionated egg lecithinase, 1.2 g; glycerol, 2.5 g; water to 100 ml. Main fatty acid components, analyzed by gas-liquid chromatography by Miss Inger Bjerkedal, were: linoleate (40%), oleate (24%), palmitate (10%), and linoleneate (7%).

dimensions remained constant. Although coronary flow was unchanged, $M\dot{V}O_2$ increased by 17% ($P < 0.025$). It should be noted that myocardial lactate uptake was essentially unchanged by the lipid infusion.

Myocardial hemodynamics and metabolism following reduced coronary flow (Group 2). Stepwise reduction of myocardial flow resulted in significant ischemia, manifested by increased MCL. A moderate ischemic increment in ventricular dimensions occurred without significant increase in end-diastolic pressure. However, a further ischemic increase in ventricular dimensions was invariably associated with increased end-diastolic pressure. The relationship was thus curvilinear, and the changes in end-diastolic myocardial chord length (EDMCL) were larger for a given change in end-diastolic presure below 10 mm Hg than at higher end-diastolic pressure (Fig. 1). Accordingly, EDMCL was a sensitive index for early myocardial ischemia, as studied in the present experiments.

Reduction of coronary flow in two steps lowered $M\dot{V}O_2$ to 80.7±3.1% and 60.5±5.6% of controls, respectively. Correspondingly, EDMCL increased by 4±11 and 11.7±1.9%. The ventricular dilatation correlated closely to changes in $M\dot{V}O_2$, as indicated in Fig. 2. In the same periods, hemodynamic parameters were unchanged or fell slightly, systolic LVP (LVSP) was reduced to 97±2.4% and 84±2.4%, dP/dt to 94±3.5% and 80±2.9%, respectively, and heart rate (HR) was unchanged. In control conditions, all animals demonstrated myocardial lactate uptake (33.0±8.4 μmoles/min). During the succeeding ischemic periods, lactate uptake fell and reversed to lactate production, demonstrating good correspondence to ventricular dilatation (Fig. 3).

Effects of FFA on myocardial hemodynamics and metabolism during ischemia (Group 3). A representative

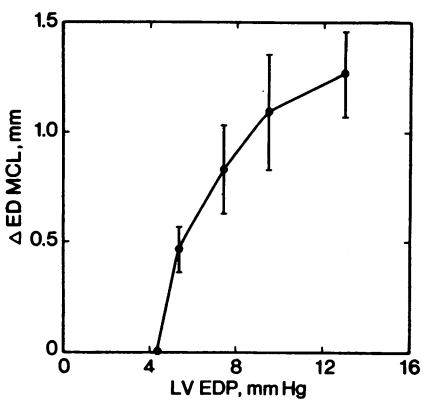

FIGURE 1 Relationship between left ventricular end-diastolic pressure (LVEDP) and increase in end-diastolic myocardial chord length (ΔEDMCL) during stepwise reduction of left coronary flow. (Mean values ±SEM in nine dogs.)

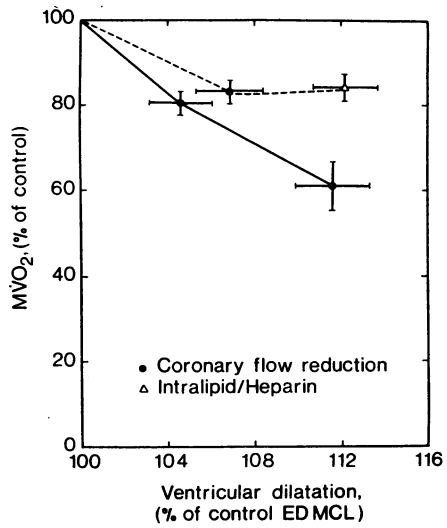

FIGURE 2 Left ventricular dilatation and oxygen consumption during stepwise reduction of coronary blood flow (nine dogs, unbroken line) and during i.v. infusion of Intralipid/heparin emulsion with the coronary flow fixed at an ischemic level (10 dogs, dotted line). (Mean ±SEM.)

experiment is shown in Fig. 4. Reduction of coronary flow from a control of 85 ml/min to 75 ml/min established a constant ischemic dilatation and myocardial lactate production. Elevation of arterial concentrations of FFA by i.v. infusion of Intralipid/heparin emulsion was followed by further increases in MCL and lactate production, irrespective of unchanged LVP, dP/dt,

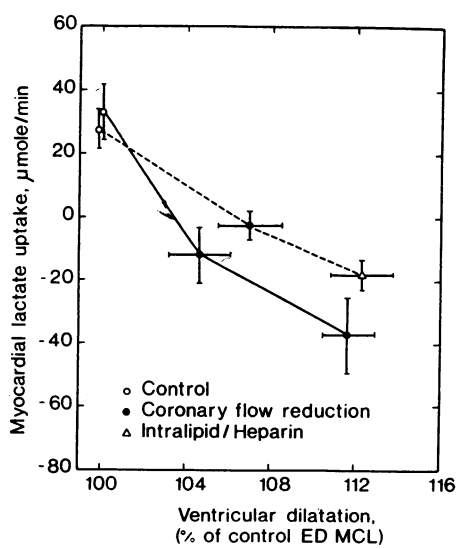

FIGURE 3 Left ventricular dilatation and lactate uptake during stepwise reduction of coronary blood flow (nine dogs, unbroken line) and during i.v. infusion of Intralipid/heparin emulsion with the coronary flow fixed at an ischemic level (10 dogs, dotted line). (Mean ±SEM.)

TABLE I

Effect of Free Fatty Acids on Myocardial Hemodynamics and

Dog No.	LVSP		dP/dt		SV		HR		EDMCL	
	C	F	C	F	C	F	C	F	C	F
	mm Hg		*mm Hg·sec⁻¹*		*ml*		*min⁻¹*		*mm*	
101	110	115	1500	1800	13.0	12.9	176	176	10.60	10.55
102	98	100	1100	1200	21.8	20.0	138	150	11.55	11.40
103	127	130	2000	2000	10.0	10.0	200	198	11.60	11.60
104	125	125	1400	1400	16.7	15.7	110	105	8.65	8.65
Mean	115	118	1500	1600	15.4	14.7	156	157	10.60	10.60
±SEM	6.8	6.6	125	183	2.5	2.1	19.9	20.0	0.69	0.67
P (C–F)										

CF, coronary shunt flow; a, artery; u, myocardial uptake; C, control; F, Intralipid infusion.
* *P* values < 0.05.

aortic flow, and $M\dot{V}O_2$. Conversely, when arterial FFA concentration was lowered by glucose/insulin, MCL and lactate production tended to return to levels observed before start of lipid infusion. Release of the coronary artery clamp was followed by return to control values of coronary flow and ventricular dimensions within 4 min.

Similar results were obtained in all animals. Hemodynamic and metabolic data are given in Tables II and III, respectively.

Before Intralipid/heparin infusion, $M\dot{V}O_2$ was reduced on an average to 82±2.6% of controls by partial clamping of the coronary shunt. Ischemia was manifested by depressed lactate utilization, demonstrated by lactate production in most animals. Simultaneously, EDMCL increased by an average of 7.1±1.4%. Infusion of Intralipid/heparin increased arterial plasma concentrations of FFA from 359±47 μEq/l to 3688±520 μEq/l and myocardial uptake of FFA more than three times. The lipid regimen had virtually no effect on $M\dot{V}O_2$ (Fig. 2). However, raised plasma concentrations of FFA were invariably associated with further dilatation of the left ventricle in all animals examined. The ultimate dilatation attained was 12.2±1.5% of control. The increase in ventricular dimensions started 30–60 sec after

TABLE II

Effects of Free Fatty Acids on Myocardial

Dog No.	LVSP			LVEDP			dP/dt			SV		
	C	I	F	C	I	F	C	I	F	C	I	F
	mm Hg			*mm Hg*			*mm Hg·sec⁻¹*			*ml*		
201	125	120	115	4	11	11	1400	1500	1500	20.7	16.9	16.9
202	110	105	112	3	7	10	1400	1200	1100	15.3	11.6	11.6
203	135	135	135	6	9	12	1600	1600	2100	6.5	5.1	4.2
204	100	100	105	6	7	9	1300	1400	1400	13.9	13.9	13.5
205	115	110	110	9	11	14	1400	1400	1400	24.2	22.7	20.6
206	125	120	120	3	3	4	2000	1500	1500	—	—	—
207	100	100	95	4	5	5	1000	1000	1000	14.7	14.7	14.7
208	115	105	110	4	5	12	1300	1300	1300	25.2	24.0	24.6
209	120	120	125	3	7	13	1800	1600	1800	9.5	9.5	9.5
210	125	105	105	2	8	10	1500	1000	900	15.7	15.0	12.5
Mean	117	112	113	4.4	7.3	10.0	1470	1350	1400	16.2	14.8	14.2
±SEM	3.6	3.6	3.6	0.7	0.8	1.0	88	70	115	2.1	2.0	2.0
P (G–I)		*			*						*	
P (I–F)						*						

C, control; I, ischemia; F, Intralipid infusion during ischemia; LVEDP, left ventricular end-diastolic pressure; CF, coronary shunt flow; CP, coronary pressure.
* *P* values < 0.05; ** *P* values < 0.001.

Metabolism during Unrestricted Coronary Flow

CF		MVO_2		Lactate a		Lactate u		FFA a		FFA u	
C	F	C	F	C	F	C	F	C	F	C	F
$ml \cdot min^{-1}$		$ml \cdot min^{-1}$		$mmole \cdot liter^{-1}$		$\mu mole \cdot min^{-1}$		$\mu Eq \cdot liter^{-1}$		$\mu Eq \cdot min^{-1}$	
75	78	8.2	9.2	1.50	1.89	26.3	42.7	348	2840	2.0	11.7
85	90	8.0	9.8	0.88	0.77	28.0	29.7	335	1360	4.8	8.9
68	70	5.0	5.6	1.00	1.08	15.4	10.5	419	1320	7.5	12.4
50	50	5.2	6.2	1.46	1.51	10.1	7.6	293	2920	2.2	5.7
70	72	6.6	7.7	1.21	1.31	20.0	22.6	349	2110	4.1	9.7
7.4	8.4	0.87	1.05 *	0.16	0.25	4.31	8.30	26	445 *	1.3	1.5 *

start of infusion of the lipid emulsion, and a plateau was usually reached within 10 min. In correspondence with the ventricular dilatation induced by FFA, myocardial lactate production increased from an average value of 3.2±5.2 to 20.5±5.6 μmoles/min. A relationship was demonstrable between lactate production and ventricular dilatation similar to that obtained by coronary constriction alone (Fig. 3), suggesting that increased myocardial FFA supply was followed by aggravation of the ischemic condition. LVSP, dP/dt, and HR remained virtually unchanged by the lipid infusion, and could not explain the above-mentioned changes induced by FFA elevation. SV remained constant, or was slightly reduced.

Substitution of the lipid infusion with glucose and insulin effected a 50% reduction in plasma-FFA concentrations after 15 min (Table IV). The fall in FFA was associated with a reversal of the ventricular dilatation approaching the dimensions obtained before lipid infusion. Concomitantly, myocardial lactate production was reduced. The reversal of the ischemic changes was not related to changes in $M\bar{V}O_2$. LVSP, SV, and dP/dt were unchanged, or slightly reduced.

Hemodynamics during Coronary Constriction

HR			CF			CP			EDMCL		
C	I	F	C	I	F	C	I	F	C	I	F
	min^{-1}			$ml \cdot min^{-1}$			$mm\ Hg$			mm	
180	200	203	105	75	70	110	58	58	13.35	14.30	14.42
171	169	167	75	48	48	90	45	45	10.65	11.95	12.75
231	222	231	85	75	73	110	75	75	12.25	12.65	13.15
120	118	118	75	55	55	75	45	48	10.65	10.85	11.40
150	154	162	110	90	90	105	60	60	9.25	9.95	10.20
143	140	140	135	85	85	120	45	45	8.70	9.55	9.85
150	150	150	90	65	75	70	35	35	9.25	9.50	10.45
141	160	165	120	70	60	95	58	58	9.48	10.00	10.40
200	200	200	65	50	55	105	80	90	11.90	12.50	13.50
110	105	105	50	40	38	120	50	55	8.65	10.10	10.40
160	162	164	91	65	65	100	56	57	10.41	11.14	11.64
11.6	11.7	12.3	8.3	5.3 **	5.2	5.5	4.3 **	5.1	0.52	0.52 **	0.52 **

TABLE III

Effects of Free Fatty Acids on Myocardial

Dog No.	$M\dot{V}O_2$			O_2 extr.			Lactate a			Lactate a-cs		
	C	I	F	C	I	F	C	I	F	C	I	F
	ml·min⁻¹			%			*mmole·liter⁻¹*			*mmole·liter⁻¹*		
201	12.2	10.5	10.6	81	89	84	—	—	—	—	—	—
202	8.6	6.8	6.5	67	85	84	1.50	2.54	2.70	0.36	0.16	−0.14
203	11.1	9.9	10.3	78	84	86	1.43	1.71	2.04	0.38	−0.30	−0.58
204	7.9	6.1	6.0	70	73	74	0.89	1.71	1.60	0.22	0.34	−0.07
205	10.7	10.1	10.2	74	84	85	1.20	1.25	1.44	0.32	0.06	−0.29
206	14.6	11.4	11.9	61	73	77	0.71	0.78	0.96	0.06	−0.21	−0.25
207	6.7	5.5	5.6	56	75	73	1.13	3.10	3.38	0.53	0.20	−0.09
208	14.3	10.0	9.6	75	82	84	1.62	1.71	1.58	0.56	−0.13	−0.32
209	4.8	4.5	4.4	73	87	82	1.73	2.40	3.58	0.15	−0.03	−0.10
210	6.8	5.0	5.5	77	85	79	1.53	1.23	1.34	0.22	−0.57	−1.37
Mean	9.7	7.9	8.1	71	82	81	1.17	1.64	1.86	0.31	−0.05	−0.32
±SEM	1.1	0.8	0.8	2.5	1.9	1.5	0.10	0.57	0.28	0.05	0.09	0.12
P (C–I)		*			**			*			*	
P (I–F)												*

O_2 extr., myocardial oxygen extraction; a-cs, coronary arteriovenous difference. Other abbreviations as in Tables I and II.
* *P* values < 0.05; ** *P* values < 0.001.

DISCUSSION

As in earlier studies (2–5), FFA stimulated myocardial oxygen utilization during free coronary flow without associated changes in mechanical performance of the heart. By extending the experiments to include hearts with the coronary vascular system fixed at an ischemic level, it was found that $M\dot{V}O_2$ remained unchanged during elevation of plasma FFA concentrations; however, the left ventricle was further dilated.

It is unlikely that the dilatation is related to increased preload induced by infusion of Intralipid/heparin emulsion, since identical infusion rates of blood did not result in dilatation of the ischemic heart, and further, SV and LVSP were unchanged by similar infusion rates of lipid during free coronary flow (Table I). In both ischemic and fully oxygenated hearts, coronary blood flow was essentially unaffected by the concentration at which FFA was presented to the heart (Tables I and III), thus eliminating any primary effect on coronary hemodynamics.

Evidence has been provided that FFA inhibits glycolysis in perfused rat hearts (18). Energy derived from a nonoxygen dependent metabolism might thereby be limited, and possibly accounts for the dilatation observed despite unchanged $M\dot{V}O_2$. However, inhibition of glycolysis has not been confirmed in oxygen-limited preparations (19), and the present finding of a close correlation between ventricular dilatation and lactate excretion —whether the dilatation was caused by a greater re-

TABLE IV

Effects of Free Fatty Acids and Glucose/Insulin Infusion on Myocardial

Dog No.	LVSP			dP/dt			SV			HR		
	I	F	G	I	F	G	I	F	G	I	F	G
	mm Hg			*mm Hg·sec⁻¹*			*ml*			*min⁻¹*		
203	135	135	125	1900	2100	1900	11.5	8.3	9.7	222	231	231
208	105	110	95	1300	1300	1300	23.9	24.6	23.9	160	171	162
209	120	125	125	1600	1800	1600	9.5	9.5	9.0	200	200	200
210	105	105	100	1000	900	900	15.0	12.5	14.2	105	105	105
Mean	116	119	114	1450	1525	1425	15.0	13.7	14.2	172	177	174
±SEM	7.2	6.9	6.2	194	266	214	3.2	3.7	3.4	25.7	26.9	27.1

I, control ischemia; F, Intralipid infusion; G, glucose/insulin infusion. Other abbreviations as in Tables I and II.

Metabolism during Coronary Constriction

Lactate			FFA								
u			a			a-cs			u		
C	I	F	C	I	F	C	I	F	C	I	F
$\mu mole \cdot min^{-1}$			$\mu Eq \cdot liter^{-1}$			$\mu Eq \cdot liter^{-1}$			$\mu Eq \cdot min^{-1}$		
—	—	—	290	204	3920	99	64	50	7.3	3.4	2.5
27.0	7.7	− 6.7	348	383	5690	43	122	—	2.0	3.8	—
32.2	−22.0	−40.7	209	283	2120	35	87	270	1.9	4.3	13.0
16.5	18.7	− 3.8	435	348	4020	130	174	290	7.4	5.6	9.5
35.2	5.4	−26.1	—	340	4150	—	127	455	—	8.0	28.2
8.1	−17.8	−21.2	708	674	3380	208	239	795	17.4	11.9	47.3
47.7	13.0	− 8.8	437	333	3565	124	83	565	7.7	3.8	28.4
67.2	− 9.1	−19.2	168	188	1930	30	62	505	2.3	2.8	19.1
10.4	− 1.5	− 5.5	628	545	1404	49	63	126	2.5	2.5	5.5
11.0	−22.8	−52.1	419	293	6700	64	42	90	2.0	1.1	2.1
27.3	− 3.2	−20.5	404	359	3688	87	106	349	5.6	4.7	15.6
6.6	5.2	5.6	59	47	520	17.6	19.3	83.0	1.7	0.9	5.0
	*										
		**			**			*			*

duction in coronary flow or by elevation of plasma concentrations of FFA—suggests that in this setting inhibition of glycolysis by FFA is of no importance.

Since LVSP and SV are maintained with a larger end-diastolic volume, a reduction in myocardial contractility is implied. The data therefore strongly suggest a depressive effect of FFA on the ischemic heart.

High concentrations of unbound FFA in vitro have been shown to inhibit enzyme activities due to detergent properties of FFA (20), and are highly toxic to the perfused heart (21); however, it is uncertain whether this applies to in vivo conditions, assuming sufficient protein binding capacity for FFA. Myocardial performance in the hypoxic papillary muscle is depressed equally by high concentrations of nonmetabolizable and metabolizable FFA (10). On the other hand, the increase in $M\dot{V}O_2$ obtained with albumin-FFA in the oxygenated heart seems to be attributable solely to metabolizable FFA (22). Thus, the effect of FFA on $M\dot{V}O_2$ appears to be related to its oxidation, while the depression of myocardial performance is not necessarily so.

It may be asked whether FFA induces dilatation of the ischemic heart through a mechanism similar to that which increases oxygen consumption in the nonischemic heart (2), or through a primary depressive effect on myocardial contractility. Henderson, Craig, Gorlin and Sonnenblick (11) found a depression of both $M\dot{V}O_2$ and mechanical performance in isolated oxygenated perfused rat hearts when high concentrations of FFA were added to the perfusate, and could not exclude a primary de-

Hemodynamics and Metabolism during Ischemia

EDMCL			CF			$M\dot{V}O_2$			$Lactate_a$			FFA_a		
I	F	G	I	F	G	I	F	G	I	F	G	I	F	G
mm			$ml \cdot min^{-1}$			$ml \cdot min^{-1}$			$\mu mole \cdot min^{-1}$			$\mu Eq \cdot liter^{-1}$		
12.65	13.15	12.90	75	73	70	9.9	10.3	9.6	−22.0	−40.7	−17.6	283	2120	451
10.00	10.40	10.18	70	60	50	10.0	9.6	8.8	− 9.1	−19.2	—	188	1930	—
12.50	13.50	12.80	50	55	50	4.5	4.4	4.7	− 1.5	− 5.5	4.0	545	1404	922
10.10	10.40	9.95	40	38	40	5.0	5.5	5.6	−22.8	−52.1	−14.4	293	6700	3960
11.31	11.86	11.45	59	57	53	7.4	7.5	7.2	−13.9	−29.4	− 9.3	327	3039	1778
0.72	0.85	0.81	8.3	7.2	6.3	1.5	1.5	1.2	5.2	10.5	7.2	76	1230	1110

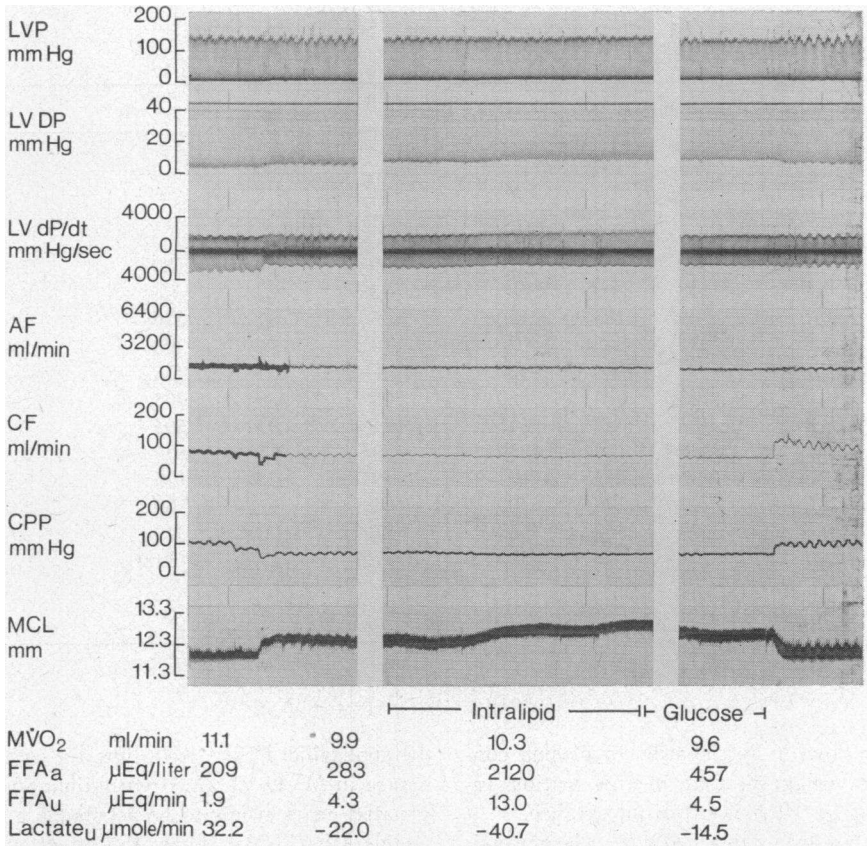

FIGURE 4 Hemodynamic and metabolic changes after graded coronary flow reduction in a representative dog heart. During a fixed coronary constriction plasma concentration of FFA was increased by i.v. infusion of Intralipid/heparin. Infusion of glucose 5.5% and insulin was initiated after 20 min of coronary ischemia, replacing the lipid infusion. AF, aortic flow; CF, flow in the shunt to the left coronary artery; CPP, pressure in the shunt distal to the clamp; FFA_a, arterial plasma concentration of FFA; FFA_u, myocardial uptake of FFA; $Lactate_u$, myocardial uptake of lactate. Paper speed 0.25 mm/sec.

pressive effect on the heart. In contrast, results obtained during free coronary flow in the present and previous studies in more intact preparations (2) provide strong evidence in favor of a primary stimulatory effect of FFA on myocardial oxygen requirement, with no impairment of cardiac contractility. However, Henderson et al. (11) agree that their hearts might have been oxygen-limited.

$M\dot{V}O_2$ was unchanged by lipid infusion when the coronary vascular system was fixed at an ischemic flow level in the present experiments. However, the dilatation following FFA elevation was associated with increased lactate excretion. This suggests increased anaerobic metabolism, since myocardial lactate uptake during unrestricted coronary flow was not influenced by high levels of FFA. Similar relationships were otbained between ventricular dilatation and lactate excretion during stepwise reductions in flow and $M\dot{V}O_2$ by constriction of the coronary shunt. The FFA-induced dilatation might therefore be related to hypoxia relative to increased oxygen requirement.

Furthermore, stepwise reductions in coronary blood flow—which had only small effects on LVSP and SV—demonstrated a close and inverse relationship between $M\dot{V}O_2$ and the increase in ventricular dimensions. By comparison, the magnitude of dilatation associated with high concentrations of arterial FFA at a fixed coronary ischemia, corresponded to that obtained from a reduction in $M\dot{V}O_2$ of about 17% (Fig. 2). This figure is close to the rise in $M\dot{V}O_2$ when uptake of FFA is similarly increased during free coronary flow, as seen in this and in previous studies (2, 5, 22). Accordingly, the present experiments suggest that high concentrations of plasma FFA increase the oxygen requirement equally in the oxygenated and the oxygen-limited heart, indicating a similar mechanism for the effect of FFA under both

conditions. Whether this mechanism is similar to the clearly toxic effect exerted by unbound FFA remains to be studied.

The effect of high levels of triglycerides could not be separately tested in our preparation; however, Intralipid given without heparin did not increase $M\dot{V}O_2$ during free coronary flow in the intact dog (5), and is therefore hardly responsible for the observed dilatation during ischemia.

The mechanism for the FFA-stimulated increase in oxygen requirement has not yet been established. Although triglyceride formation is an energy-requiring process, and triglyceride synthesis is stimulated by increased availability of FFA (23)—even more so during hypoxia (23, 24)—the amount of oxygen required for esterification of excess FFA (25) amounts to less than 15% of the actual increase in oxygen requirement observed during enhanced FFA uptake.

It is generally accepted that the coupling of oxidative phosphorylation is regulated by cellular metabolites. In vitro studies have shown that fatty acids uncouple respiratory chain phosphorylation (26–28), and could thus explain the increase in oxygen requirement. In addition, evidence has been provided that excess FFA may be oxidized by mechanisms not coupled to ATP formation (29–31). If the latter were effective, adenosine might remain unchanged. Since adenosine is a mediator of coronary flow regulation (32), this might explain why FFA increases $M\dot{V}O_2$ mainly by increasing coronary arteriovenous oxygen extraction during free flow. In contrast, dinitrophenol—an uncoupler of oxidative phosphorylation—increases $M\dot{V}O_2$ by increasing flow, while oxygen extraction is unchanged (33).

Although conflicting results have been obtained in assessing the efficacy of glucose in the treatment of acute coronary ischemia (34–36), evidence has been provided of prolonged survival of ischemic tissue during glucose treatment (37–39). The rationale suggested for using glucose or combinations of glucose, K^+, and insulin in treating myocardial infarction has been to supply adequate glucose to the heart, thereby obtaining energy from anaerobic breakdown of glucose (37, 38, 40), reverse potassium loss from the heart, and maintain membrane stability (41, 42). It is known that glucose/insulin accelerates triglyceride formation (19) and decreases release of FFA from adipose tissue (43). When glucose/insulin was substituted for Intralipid infusion in our experiments, arterial concentrations of FFA fell by 50% after 15 min, probably indicating a decrease in cellular concentration of FFA. A corresponding reversal of the ischemic dilatation and lactate production could be demonstrated. The present findings suggest that the effect of glucose on the ischemic heart is at least partly related to the depression of intracellular levels of FFA, and thus to a reduction in the myocardial oxygen requirement.

Patients with ischemic heart disease display high concentrations of plasma FFA (7, 8); average concentrations, however, were less than those obtained with Intralipid/heparin. On the other hand, individual experiments showed that ventricular dilatation of the ischemic heart could be achieved with similar FFA levels to those encountered in patients. Further increase of lipid infusion in these experiments was not followed by additional dilatation. Thus, our study suggests that increased concentrations of plasma FFA occurring in a patient with acute coronary occlusion might extend the area of relative ischemia and cause impaired left ventricular function. Attention is also drawn to the potentially deleterious effects of administering catecholamines to patients with acute myocardial infarction, owing to the potent lipolytic effect of these agents (22). Clinically, it would be of interest to inhibit the lipolytic effect induced by increased sympatho-adrenal activity so as to reduce the ultimate size of the infarction.

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REFERENCES

1. Maroko, P. R., J. K. Kjekshus, B. E. Sobel, T. Watanabe, J. W. Covell, J. Ross, Jr., and E. Braunwald. 1971. Factors influencing infarct size following experimental coronary artery occlusions. *Circulation.* **43**: 67.
2. Mjøs, O. D. 1971. Effect of free fatty acids on myocardial function and oxygen consumption in intact dogs. *J. Clin. Invest.* **50**: 1386.
3. Mjøs, O.D. 1971. Effect of inhibition of lipolysis on myocardial oxygen consumption in the presence of isoproterenol. *J. Clin. Invest.* **50**: 1869.
4. Mjøs, O. D. 1971. Effect of isoproterenol, glucagon and calcium on myocardial oxygen consumption in intact dogs. A comparative study. *Scand. J. Clin. Lab. Invest.* **28**: 127.
5. Mjøs, O. D. 1971. Free fatty acids and oxygen consumption in dogs. *Scand. J. Clin. Lab. Invest.* **28**: 121.
6. Mjøs, O. D., and J. Kjekshus. 1971. Increased local metabolic rate by free fatty acids in the intact dog heart. *Scand. J. Clin. Lab. Invest.* **28**: 389.
7. Kurien, V. A., and M. F. Oliver. 1966. Serum-free-fatty-acids after acute myocardial infarction and cerebral vascular occlusion. *Lancet.* **II**: 122.
8. Oliver, M. F., V. A. Kurien, and T. W. Greenwood. 1968. Relation between serum-free-fatty-acids and arrhythmias and death after acute myocardial infarction. *Lancet.* **I**: 710.

9. Kurien, V. A., P. A. Yates, and M. F. Oliver. 1971. The role of free fatty acids in the production of ventricular arrhythmias after acute coronary artery occlusion. *Eur. J. Clin. Invest.* **1**: 225.
10. Henderson, A. H., A. S. Most, W. W. Parmley, R. Gorlin, and E. H. Sonnenblick. 1970. Depression of myocardial contractility in rats by free fatty acids during hypoxia. *Circ. Res.* **26**: 439.
11. Henderson, A. H., R. J. Craig, R. Gorlin, and E. H. Sonnenblick. 1970. Free fatty acids and myocardial function in perfused rat hearts. *Cardiovasc. Res.* **4**: 466.
12. Bugge-Asperheim, B., S. Leraand, and F. Kiil. 1969. Local dimensional changes of the myocardium measured by ultrasonic technique. *Scand. J. Clin. Lab. Invest.* **24**: 361.
13. Meng, H. C., and B. Edgren. 1963. Source of plasma free fatty acids in dogs receiving fat emulsion and heparin. *Amer. J. Physiol.* **204**: 691.
14. Aukland, K. 1962. Spectrophotometric determination of hemoglobin oxygen saturation in small blood samples. *Scand. J. Clin. Lab. Invest.* **14**: 533.
15. Hohorst, H.-J. 1962. L-(+)-Lactat. Bestimmung mit Lactat-Dehydrogenase und DPN. *In* Methoden der enzymatischen Analyse. H.-U. Bergmeyer, editor. Verlag-Chemie GmbH, Weinheim.
16. Dole, V. P. 1956. A relation between non-esterified fatty acids in plasma and the metabolism of glucose. *J. Clin. Invest.* **35**: 150.
17. Trout, D. L., E. H. Estes, and S. J. Friedberg. 1960. Titration of free fatty acids of plasma: a study of current methods and a new modification. *J. Lipid Res.* **1**: 199.
18. Shipp, J. C., L. M. Opie, and D. Challoner. 1961. Fatty acid and glucose metabolism in the perfused heart. *Nature (London).* **189**: 1018.
19. Randle, P. J., P. B. Garland, C. N. Hales, and E. A. Newsholme. 1963. The glucose fatty-acid cycle, its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet.* **II**: 785.
20. Pande, S. V., and J. F. Mead. 1968. Inhibition of enzyme activities by free fatty acids. *J. Biol. Chem.* **243**: 6180.
21. Severeid, L., W. E. Connor, and J. P. Long. 1969. The depressant effect of fatty acids on the isolated rabbit heart. *Proc. Soc. Exp. Biol. Med.* **131**: 1139.
22. Challoner, D. R., and D. Steinberg. 1966. Effect of free fatty acid on the oxygen consumption of perfused rat heart. *Amer. J. Physiol.* **210**: 280.
23. Evans, J. R. 1964. Importance of fatty acid in myocardial metabolism. *Circ. Res.* **15** (Suppl.): II-96.
24. Scheuer, J., and N. Brachfeld. 1966. Myocardial uptake and fractional distribution of palmitate-1-C^{14} by the ischemic dog heart. *Metabolism.* **15**: 945.
25. Ball, E. G. 1965. Some energy relationships in adipose tissue. *Ann. N. Y. Acad. Sci.* **131**: 225.
26. Pressman, B. C., and H. A. Lardy. 1956. Effect of surface active agents on the latent ATPase of mitochondria. *Biochim. Biophys. Acta.* **21**: 458.
27. Borst, P., J. A. Loos, E. J. Christ, and E. C. Slater. 1962. Uncoupling activity of long-chain fatty acids. *Biochim. Biophys. Acta.* **62**: 509.
28. Hittelman, K. J., and O. Lindberg. 1970. Fatty acid uncoupling in brown fat mitochondria. *In* Brown Adipose Tissue. O. Lindberg, editor. American Elsevier Publishing Co., Inc., New York. 245.
29. Challoner, D. R., and D. Steinberg. 1966. Oxidative metabolism of myocardium as influenced by fatty acids and epinephrine. *Amer. J. Physiol.* **211**: 897.
30. Challoner, D. R. 1968. Evidence for uncoupled respiration in thyrotoxic and epinephrine-stimulated myocardium. *Amer. J. Physiol.* **214**: 365.
31. Rossi, C. R., and D. M. Gibson. 1964. Activation of fatty acids by a guanosine triphosphate-specific thiokinase from liver mitochondria. *J. Biol. Chem.* **239**: 1694.
32. Berne, R. M. 1963. Cardiac nucleotides in hypoxia: possible role in regulation of coronary blood flow. *Amer. J. Physiol.* **204**: 317.
33. Scott, J. C., M. Gold, A. A. Bechtel, and J. J. Spitzer. 1968. Influence of 2,4-dinitrophenol on myocardial metabolism and hemodynamics. *Metabolism.* **17**: 370.
34. Mittra, B. 1965. Potassium, glucose, and insulin in treatment of myocardial infarction. *Lancet.* **II**: 607.
35. Medical Research Council Working-Party. 1968. Potassium, glucose and insulin treatment for acute myocardial infarction. *Lancet.* **II**: 1355.
36. Sodi-Pallares, D., J. Ponce de León, A. Bisteni, and G. A. Medrano. 1969. Potassium, glucose, and insulin in myocardial infarction. *Lancet.* **I**: 1315.
37. Winbury, M. M. 1956. Influence of glucose on contractile activity of papillary muscle during and after anoxia. *Amer. J. Physiol.* **187**: 135.
38. Yang, W. C. 1963. Anaerobic functional activity of isolated rabbit atria. *Amer. J. Physiol.* **205**: 781.
39. Weissler, A. M., F. A. Kruger, N. Baba, D. G. Scarpelli, R. F. Leighton, and J. K. Gallimore. 1968. Role of anaerobic metabolism in the preservation of functional capacity and structure of anoxic myocardium. *J. Clin. Invest.* **47**: 403.
40. Owen, P., M. Thomas, and L. Opie. 1969. Relative changes in free-fatty-acid and glucose utilisation by ischemic myocardium after coronary-artery occlusion. *Lancet.* **I**: 1187.
41. Sodi-Pallares, D., A. Bisteni, G. A. Medrano, M. R. Testelli, and A. de Micheli. 1963. The polarizing treatment of acute myocardial infarction. Possibility of its use in other cardiovascular conditions. *Dis. Chest.* **43**: 424.
42. Regan, T. J., M. A. Harman, P. H. Lehan, W. M. Burke, and H. A. Oldewurtel. 1967. Ventricular arrhythmias and K^+ transfer during myocardial ischemia and interventions with procaine amide, insulin, or glucose solution. *J. Clin. Invest.* **46**: 1657.
43. Carlson, L. A. 1965. Inhibition of the mobilization of free fatty acids from adipose tissue. *Ann. N. Y. Acad. Sci.* **131**: 119.