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THE EFFECT OF PALMITATE AND LACTATE ON MECHANICAL PERFORMANCE AND METABOLISM OF CAT AND RAT MYOCARDIUM

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SUMMARY

1. Fourteen isolated ejecting hearts were perfused with a suspension of red cells in Tyrode solution.

2. In five hearts comparison was made between glucose alone as substrate and glucose plus free fatty acid (palmitate). In five hearts the effect of additional lactate was studied. In the remaining hearts no substrate changes were made (controls).

3. There were only transient changes in cardiac output of the hearts (at fixed mean aortic pressure) when the perfusion media were switched from one to another.

4. There were no consistent steady-state changes in myocardial oxygen consumption, mean external power, efficiency, cardiac output or coronary blood flow associated with any of the changes in substrate consumption. Thus we were unable to confirm an increase in oxygen consumption and decrease in efficiency associated with either free fatty acid or lactate as substrates.

5. Isolated rat trabeculae were deprived of exogenous substrate; their mechanical performance remained constant for approximately 10 min. Subsequent deterioration was restored by any of the three exogenous substrates.

6. We conclude that there is no oxygen wasting effect of these substrates as has previously been postulated, nor any deleterious effect of changing exogenous or endogenous carbohydrate or lipid substrate.

INTRODUCTION

Myocardial oxygen consumption may depend on the substrate available to the heart. For instance, Willebrands & van der Veen (1967) showed in isolated rat hearts

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TABLE 1

Reference	Preparation and perfusion fluid	O_2 supply conditions	Substrate control	Concentrations experimental	Results
Experiments on isolated hearts Challoner & Steinberg, 1966	Rat Langendorff Krebs buffer	Low O_2 content perfusion	Albumin 0·5 % FFA 0·025	Albumin 0·5 % FFA up to 0·74 μequiv/ml.	50 % increase in MVO_2
Willebrands & van der Veen, 1967	Rat Langendorff bicarbonate buffer	Low O_2 content perfusion	Glucose 11 mM	(*a*) Albumin 1·5 % +FFA 1 mM (*b*) Lactate 10 mM	(*a*) and (*b*) increase in MVO_2
Henderson *et al.* 1970*a*	Rat Langendorff Krebs buffer	Low O_2 content perfusion	Glucose 5 mM FFA 0·15 mM	Linoleate 1·75 mM +albumin 0·3 mM	Decrease in MVO_2 Increase of MVO_2/TTI ratio
Experiments on perfused heart *in situ* Liedtke *et al.* 1978	Right and left main coronary arteries cannulated and perfused with blood in swine	(*a*) Normal (*b*) Ischaemic	FFA 340 μM	FFA increased 3–5 times	(*a*) and (*b*) increase in MVO_2
Experiments on intact dogs Mjøs, 1971*a*	Nembutal anaesthesia closed chest. Blood	Normal	FFA 488 μequiv/l.	FFA 3105 μequiv/l. by intralipid +heparin	Increase in MVO_2 from 8·6 to 10·7 ml./100 g. min due increase in A-V O_2 difference. No change in coronary flow or cardiac performance
Mjøs, 1971*b*	Nembutal anaesthesia closed chest. Blood	Normal	FFA 434 μM	FFA 3547 μM by intralipid +heparin	Increase in MVO_2 by 19 %
Mjøs & Kjekhus 1971	Open chest. Blood	Normal	FFA 450 μM	FFA 3000 μM	Increase in myocardial heat production
Most *et al.* 1973	Closed chest. Blood	Normal	Normal	FFA 1900 μM	No increase in MVO_2
Experiments in humans Regan *et al.* 1961	Convalescent from non-cardiac illness	Normal	Fasting	Post-prandial optical density of plasma over 0·3 units	In unpaired comparison, MVO_2 decreased with lipaemia. Restored to normal by the heparin
Rogers *et al.* 1977	Normal man	Normal	FFA 713 μM	FFA 2668 μM	Directly measured MVO_2 unchanged. No change in cardiac performance

that lactate increases the amount of oxygen consumed by the heart. It has also been proposed that when plasma free fatty acid (FFA) levels are elevated, myocardial oxygen consumption may increase. However, there is conflicting evidence concerning this proposal (Table 1). Apart from contradictory evidence within one species, there appear to be species differences. If it is assumed that the amount of external work remains constant, such increases in oxygen consumption imply a decrease in mechanical efficiency of the heart.

The purpose of the present study was to re-investigate the problem of an effect on myocardial oxygen consumption by free fatty acids and lactate in the cat under controlled conditions. The problem has not been studied previously in the cat. In order to avoid the complicating factors which, in the intact animal, can interfere with the effects of the substances studied, an isolated ejecting cat heart preparation was used. To avoid inadequate oxygen supply, red blood cells were added to the perfusion fluid. In order to study the effect of exogenous substrate withdrawal, separate experiments were performed in isolated rat trabeculae.

METHODS

Preparations

Male cats, anaesthetized with sodium pentobarbitone (45 mg/kg I.P.) were artificially ventilated. The thorax was opened through a mid-sternal incision; the heart was isolated and subsequently connected to the perfusion apparatus (Fig. 1, Elzinga & Westerhof, 1980).

The isolated heart set-up consisted of two large reservoirs, with a different perfusion fluid contained in each. From the reservoir of choice (selected by using stopcocks a and b in Fig. 1), perfusate was passed under pressure through a filter to fill the left atrial supply vessel. The height of the perfusion fluid was kept constant by an overflow system. Overflowing fluid was pumped back to the reservoir.

The left ventricle ejected into a hydraulic model of the arterial input impedance of the cat, (Westerhof, Elzinga & Sipkema, 1971). This model consisted of two resistors and a compliance. The resistors were made up from an assembly of narrow conduits. The compliance consisted of a given volume of air. The value of the resistor modelling the systemic peripheral resistance was adjusted by means of a slide which varied the number of conduits open to the circulation. The slide was served by a motor with feed-back control to keep mean aortic pressure at a given level.

Cardiac output was returned to the appropriate reservoir or collected over a given time in a beaker. Coronary venous flow was pumped out by the right ventricle. This amount of fluid, which had perfused the myocardium, was not recirculated.

The fluid in the reservoir was warmed with a glass coil suspended in the perfusate through which water at 42 °C was pumped when the temperature fell below 38·5 °C. Temperature differences measured during the experiments were less than 0·5 °C, regardless of the reservoir used. Switching from one reservoir to the other caused a transient effect on temperature in the supply vessel but not in the circulation.

Isolated rat trabeculae were studied by the methods fully described by ter Keurs, Rijnsburger, van Heunigen & Nagelsmit (1980). The dimensions of the trabeculae were within the following limits: thickness 65–95 μm, width 150–300 μm, length 2000–2500 μm.

Perfusion fluids and protocols

In three groups of experiments on isolated ejecting cat hearts (see below), three different perfusate compositions were employed:

(1) Tyrode solution (Na^+, 149 mM; K^+, 4·7 mM; Cl^-, 138 mM; Ca^{2+}, 1·35 mM; Mg^{2+}, 1·05 mM; HCO_3^-, 20·3 mM; $H_2PO_4^-$, 0·42 mM; glucose, 11·1 mM, mixed with washed bovine erythrocytes (final hematocrit 25, final blood glucose 7 mM) and 1 g% fat-free albumin (Sigma).

(2) The same perfusate as described under (1) with the 1% albumin saturated with palmitate (for this purpose bovine albumin fraction V was used).

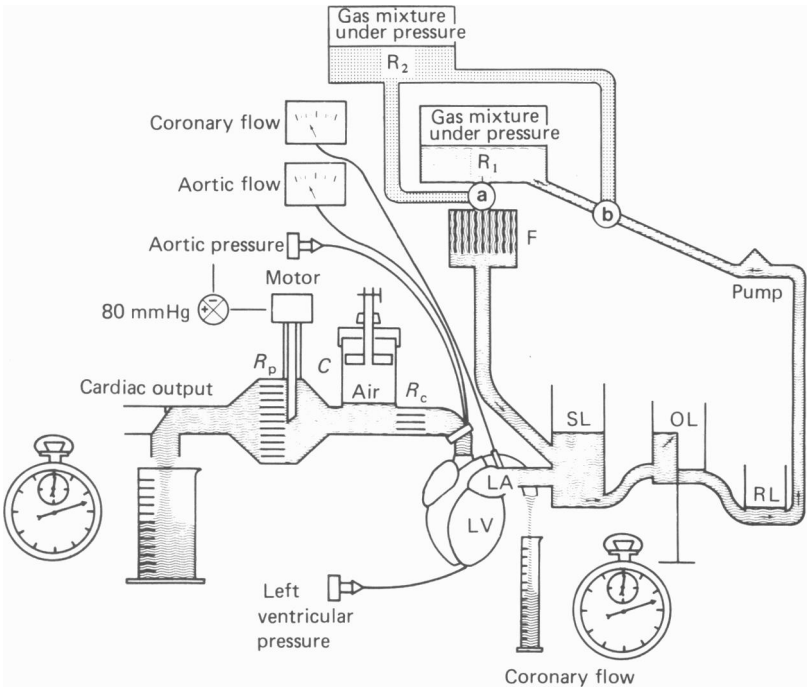

Fig. 1. Diagram of apparatus. A choice between reservoirs R_1 and R_2 could be made with stopcocks a and b. F is a filter. Left atrial (LA) filling pressure was kept constant by an overflow system (SL and OL). The left ventricle ejected into a hydraulic model of the arterial system (R_c, C and R_p). Mean aortic pressure was kept at a fixed value by adjusting the resistance R_p. The various measurements are indicated.

TABLE 2. Substrate composition and blood gas values of the arterial perfusates

	Series B (n = 5)		Series C (n = 5)	
	Control	Palmitate	Control	Lactate
Glucose (mM)	7·04±0·50	7·30±0·55	6·62±0·08	6·56±0·12
Palmitate (m-equiv/l.)	0·02±0·01	0·99±0·07	1·19±0·03	1·13±0·03
Lactate (mM)	1·02±0·14	1·16±0·09	1·09±0·19	4·58±0·20
pH	7·36±0·02	7·34±0·03	7·35±0·01	7·36±0·02
P_{CO_2} (mmHg)	29·0±1·3	28·7±1·6	31·0±1·4	30·8±1·8
P_{O_2} (mmHg)	149±48	160±64	164±31	172±28
O_2 (ml./100 ml.)	10·9±0·2	10·9±0·2	11·5±0·3	11·4±0·2

All values are given as mean±S.E. of mean.

(3) The same perfusate as under (2) with the addition of lactate to give a final concentration of 3·5–5·0 mM. The latter was added in a neutralized form. There was no pH difference between the two reservoirs.

Before the start of the experiments the perfusates were passed through an oxygenator. The gas mixture used was: 25% O_2, 5% CO_2 and 70% N_2. The two reservoirs were then filled with 6–8 l. of perfusate each. Perfusate 2 was used as the experimental solution in series B experiments and as the control solution for series C experiments. The final composition (Table 2) was slightly different in these two cases because of a small amount of admixture on recirculation. Measured substrate composition, oxygen content, P_{O_2}, P_{CO_2} and pH after oxygenation of the arterial perfusates are given in Table 2.

During all experiments mean aortic pressure was kept at 80 mmHg using the feed-back system controlling peripheral resistance (see Fig. 1). Left atrial filling pressure was adjusted at the start of each experiment to provide a cardiac output of more than 400 ml./min. It was kept constant during the remainder of the experiment. Left ventricular end-diastolic pressures varied between experiments from 2·0 to 8·9 mmHg. Heart rate was 120 beats/min in all experiments. This was achieved by left atrial pacing after damaging the sino-atrial node.

The experiments consisted of three periods of 15 min each. The first reservoir was used for the first and last 15 min periods. During the second period the heart was offered perfusate from the second reservoir. Integrated blood samples were withdrawn twice during each 15 min period and measurements made from the chart recorder 5 min after the start and at the end of each period. Since the blood samples were withdrawn over the period of coronary venous flow collection they represented aliquots of total coronary blood.

For the present study fourteen isolated heart preparations were used. The following experiments were performed.

Series A (control experiments)

In four experiments the two reservoirs contained the same perfusate. Perfusate 2 was used three times and perfusate 3 once for this purpose.

Series B (palmitate experiments)

In five experiments the effects of perfusate 1 were compared with those of perfusate 2. On four occasions the first reservoir contained perfusate 1; on one of these occasions only two 15 min periods were studied. Once the experiment started with perfusate 2.

Series C (lactate experiments)

In five experiments perfusate 2 was compared with perfusate 3. In three of the five cases perfusate 2 was used first.

Rat trabeculae

The solution used for perfusion of the heart during the dissection and the experiment had the following ionic composition (mM): Na^+, 147·4; K^+, 5·0; Cl^-, 98·5; Mg^{2+}, 1·2; $H_2PO_4^-$, 2·0; HCO_3^-, 28·0; SO_4^{2-}, 1·2; Ca^{2+}, 2·5.

Where glucose was added as substrate it was as 11·0 mM, lactate 4·0 mM, and free fatty acid as palmitate bound to albumin on a 1:7 molar basis. Albumin (bovine fraction V) 1% was added to the glucose and lactate solutions in the substrate experiments so that the colloid osmotic pressures were comparable. Mannitol, 4·0 mM, was added to the lactate and palmitate solutions to bring the osmolality up to that of the glucose solutions.

The Ca^{2+} concentration used was nominally 2·5 mM, but because of the albumin, the ionized Ca^{2+} concentration was 1·8 mM. The solutions were equilibrated with 95% CO_2:5% O_2. A membrane oxygenator was used to diminish bubble problems. The pH of the perfusion fluid was 7·30–7·40; P_{CO_2} 40 mmHg, P_{O_2} 500 mmHg. The volume of the perfusion chamber was 2·0 ml. and the flow rate kept to 2·0 ml. min^{-1}. The temperature of the fluid chamber was kept constant at 25 °C.

The stability of the entire preparation was studied by stimulating the trabeculae at 3 Hz (stimulus intensity 50% above threshold, duration 5 msec) with all three substrates present. Mechanical performance was assessed at a stimulation rate of 0·2 Hz and found to be the same for each substrate (added in a random order). The stimulation rate was then increased to 3 Hz in the absence of substrate until there was an appreciable fall in force. The stimulation rate was then returned to 0·2 Hz, and one of the substrates supplied.

Measurements, recording and analysis

Instantaneous aortic blood flow and mean coronary blood flow (outflow from the pulmonary artery canula) were measured continuously with electromagnetic flowmeters (Transflow 601; Skalar Instruments, Holland). Twice during each 15 min period (see above), output from both the left and right ventricles was collected in beakers for 30 sec, and the amount was measured by weighing.

Pressures in the aorta and left ventricle (needle through the apex) were measured with Statham P23 Db transducers via short catheters. Resonance frequencies of the pressure measuring systems were above 100 Hz. The first time derivative of left ventricular pressure (dP/dt) was obtained through analogue differentiation. The characteristics of the differentiator were: 85° phase shift at

45 Hz and 0° phase shift at 300 Hz. Instantaneous left ventricular external power was found by instantaneous multiplication of aortic blood flow and left ventricular pressure.

Arterial and venous blood samples were taken twice during each 15 min period (see above). Measurements of pH, P_{CO_2} and P_{O_2} were made with Radiometer apparatus (PHM 71 MK2; PHA 934: PHA 935). Oxygen content was determined with a LEX O_2, Con-TL (Lexington Instruments Corp).

Lactate was measured with the Boehringer Lactate Kit and glucose with the Boehringer Hexokinase-UV Kit. Free fatty acids were measured by micro-titration using an automated method of Keul, Linner & Eschenbrugch (1968).

Continuously measured variables were recorded on an Elema-Schonander ink-writing system (EMT 81) and an analogue tape recorder (SE 7000). Mean values of left ventricular external power, maximum values of left ventricular dP/dt and left ventricular end-diastolic pressures were obtained through an analogue device specially designed for the analysis of cardiovascular variables (Puls & Elzinga, 1978). This apparatus took average values of at least seven complexes for a given measurement.

The myocardial uptake rates of oxygen, lactate and free fatty acids were calculated from the Fick equation, i.e. the differences in concentration of each substance between arterial and coronary venous blood were multiplied by the coronary blood flow.

External efficiencies were calculated by taking the ratio of mean external power and the product of oxygen consumption times a calorific equivalent of 4·8 kcal per l. oxygen (see Discussion). Mean external power and oxygen consumption energy equivalent were converted to the same units for the purpose of calculating the efficiency ratio percentage.

Force developed by rat trabeculae was measured with a capacitive force transducer connected to a reactance converter (Disa 51 E01). The moving plate of the force transducer was connected through a lightweight plastic arm to the muscle clip. The sensitivity of the transducer was 0·1 V/mN, linearity 5% up to 10 mN, drift 90·2 mN/hr; resonant frequency 460 Hz, compliance 0·1 μm/mN.

Muscle length was measured by means of a variable mutual inductance displacement transducer (Metrisite 8) incorporated into a conventional servo motor system (Brush pen motor 869223). A plastic arm connected to the vertical motor axis to the steel clip. The compliance of the arm and motor was 0·8 μm/mN. Muscle length and force were recorded on a chart recorder (Brush Gould 440) and a storage oscilloscope and hard copy unit (Tektronix 5103, 613, 4631).

Measurement of sarcomere length

The muscle was contained in a glass covered chamber that enables simultaneous microscopical and laser diffraction measurements of sarcomere length using the methods of ter Keurs *et al.* (1980).

RESULTS

An example of the variables measured continuously during the cat experiments is shown in Fig. 2. Left ventricular end-diastolic pressure appears in the recording as a continuous line. Such a trace is obtained by analysis of left ventricular pressure on a beat to beat basis using the analogue device (Puls & Elzinga, 1978) employed for the analysis of other variables (see above). Fig. 2 further shows the transient effects of switching from one reservoir to another. There was a tendency for the variables to return to pre-switch values at about 1 min after the change of perfusate (Fig. 2). The reason for the transient effects following the switch is not completely clear. However, we measured a transient change in temperature (2–3 °C) in the left atrial supply vessel after the switch due to cooling of the perfusate in the leads between stopcock a and the reservoir which had not previously been in use. We therefore decided to study only the steady-state measurements made at the 5th and the 15th min of each of the three 15 min periods.

When perfusate 1 (arterial free fatty acid concentration 0·02 m-equiv/l.) was

exchanged with perfusate 2 (arterial free fatty acid concentration 0·99 m-equiv/l.) consumption of free fatty acid was higher in all five experiments when extra palmitate was offered to the hearts. Mean free fatty acid uptake during the second 15 min period changed from 0·5 m-equiv/min (low fat) to 2·0 m-equiv/min (high fat). This was associated with a fall in lactate and glucose consumption, indicating a true switch

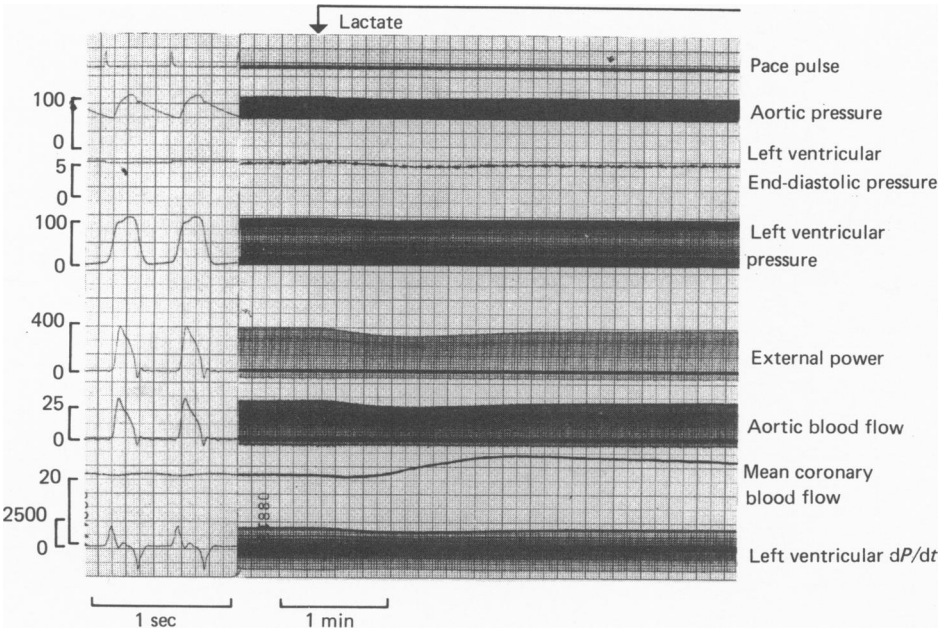

Fig. 2. Measurements before and following a switch from reservoir R_1 to R_2 (Fig. 1). In this example extra lactate was added to the perfusion fluid in R_2. The initial changes following the switch were transient only. Pressures are given in mmHg, external power in mW, aortic blood flow in ml./sec, mean coronary blood flow in ml./min and left ventricular dP/dt in mmHg/sec.

in substrate utilization and not only uptake of palmitate into lipid stores. The steady-state effects of this change in consumption pattern on cardiac mechanics and energetics are given for two individual experiments in Figs. 3 and 4. The complete data is given in Table 3. There was no consistent change in cardiac output, coronary blood flow, mean external power, oxygen consumption or efficiency. The only significant change ($P = 0{\cdot}05$) was found when the maximum rate of rise of left ventricular pressure (dP/dt_{max}) at the end of the first and second 15 min period were compared (Table 3). However, the increase in dP/dt_{max} found with the increased fat uptake was not associated with changes in oxygen consumption or efficiency (Table 3).

Exchanging perfusate 2 (low lactate 1·09 mM) with perfusate 3 (high lactate 4·6 mM) resulted in no significant changes in cardiac output, coronary blood flow or maximum rate of rise of left ventricular pressure (Fig. 3). There were also no substantial changes in mean external power, myocardial oxygen consumption or efficiency (Fig. 4). The average values of all these six variables measured just before and 15 min after the

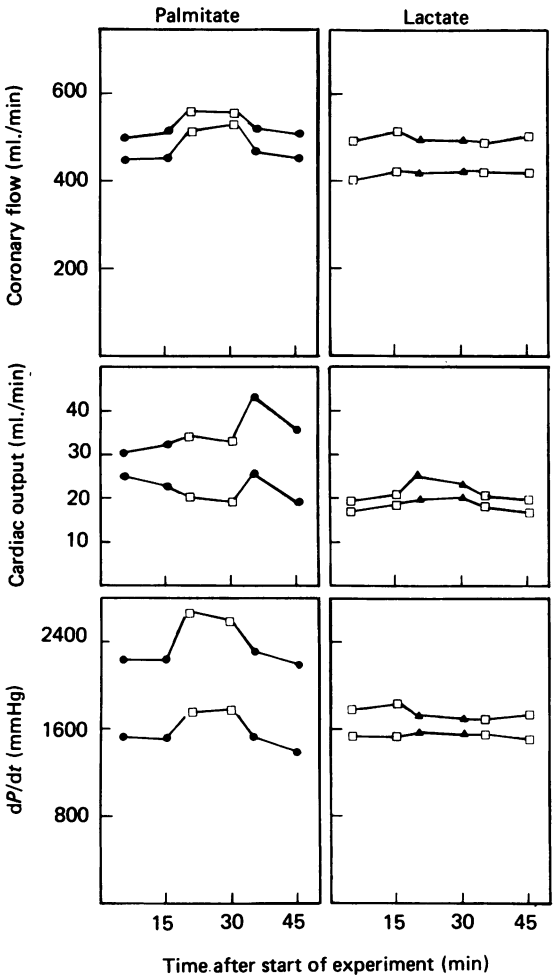

Fig. 3. Cardiac output, coronary flow and left ventricular dP/dt measured at the start and at the end of three 15 min periods. Between periods, perfusion was switched from reservoir R_1 to R_2. Two representative experiments showing the effects of palmitate (left) and lactate (right) are illustrated. Filled circles: perfusion fluid no. 1 containing glucose as substrate. Open squares: perfusion fluid no. 2 with palmitate added. Filled triangles: perfusion fluid no. 3 with lactate added. For further details of fluid composition see text.

first change of lactate concentration are given in Table 3. As in the intact dog (Drake, Haines & Noble, 1980) an increase in lactate concentration increased lactate consumption even in the presence of high free fatty acid concentration. Comparing the first and second 15 min periods, lactate consumption changed from an average of 3·18 μM/min (low lactate) to 9·21 μM/min (high lactate). When high lactate was given first (two experiments) the switch to the low lactate reservoir did not result in such a low arterial lactate (1·45 m-mole as compared to 0·85 m-mole) because of the apparatus dead space.

Lactate consumption decreased when palmitate was offered to the heart (Fig. 5

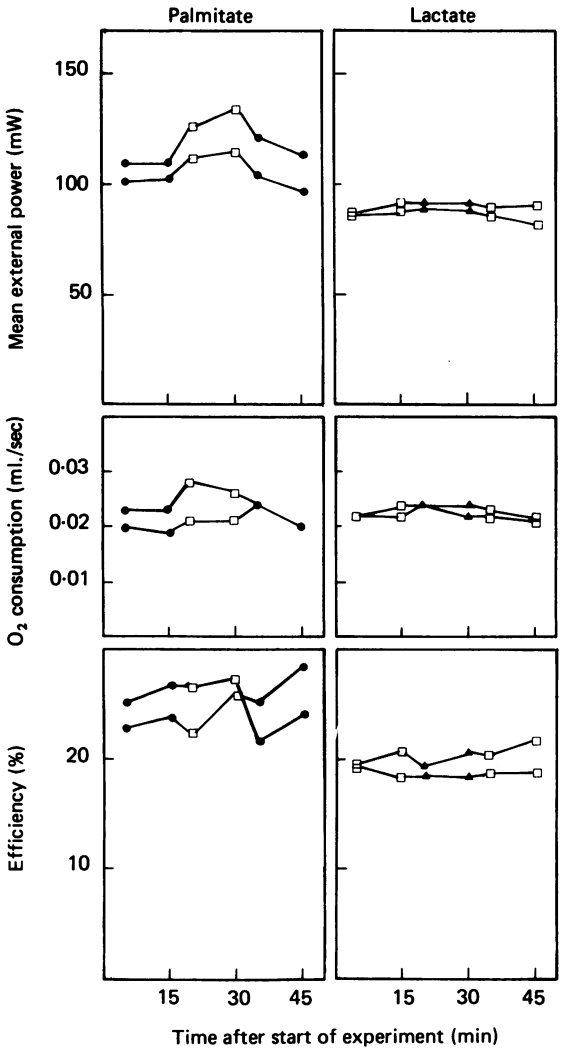

Fig. 4. The same format as Fig. 3 for mean external power, oxygen consumption and external efficiency.

left panel). This held true also in the one experiment in this series which started with high fat perfusion, although in this experiment a lactate production was found during the first and last 15 min period. This particular experiment was the only one of the entire series in which (for no apparent reason) venous lactate concentrations were higher than arterial.

In four experiments the perfusate in both reservoirs was identical. These control experiments examined the stability and variability of hearts using a single perfusate. There were no changes.

In isolated rat trabeculae, substrate withdrawal had no effect on mechanical performance for about 10 min, but deterioration took place in approximately 30 min

TABLE 3. Comparison between variables measured just before and 15 min after the first change of substrate composition

	Series B ($n = 5$)		Series C ($n = 5$)	
	Control	Palmitate	Control	Lactate
Cardiac output (ml./min)	513±25	537±14	492±17	492±20
Coronary flow (ml./min)	25·6±2·4	24·1±2·9	20·0±0·4	21·4±1·1
$dP/dt_{LV}max$ (mmHg/sec)	1764±131	2032*±170	1734±58	1788±81
Mean ext. power (mW)	115±12	126±8	107±7·2	106±7·2
O_2 consumption (ml./sec)	0·024±0·003	0·025±0·002	0·025±0·001	0·026±0·001
Efficiency (%)	24·4±0·6	24·8±0·9	21·3±1·0	20·7±0·8

All values are given as mean±S.E. of mean.
* Higher than without palmitate in all five experiments ($P = 0·05$).

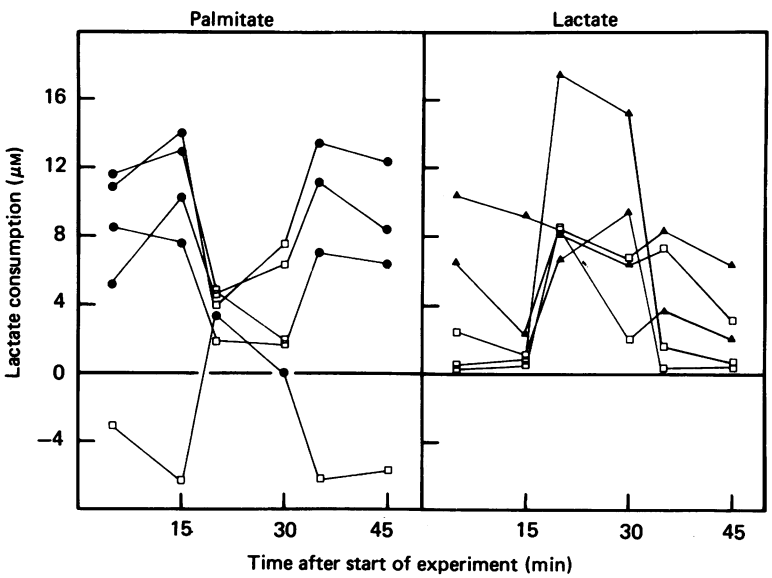

Fig. 5. Lactate uptake data for five experiments with palmitate and five with lactate. Left panel: lactate uptake fell when palmitate was given to the heart. Right panel: when the measurements at 15 and 30 min were compared, lactate uptake increased when extra lactate was present in the perfusate. Same symbols as in Figs. 3 and 4.

(Fig. 6). That this deterioration was due to substrate lack was shown by replacing exogenous substrate (Fig. 7) which resulted in similar rates of recovery regardless of whether lactate, glucose or palmitate was added.

DISCUSSION

This study shows that a change in the substrate taken up by the heart has no effect on its output at a fixed arterial pressure. There is also no effect on oxygen consumption or mechanical efficiency. This subject has elicited much interest because of the deleterious effect of free fatty acid in myocardial hypoxia and ischaemia

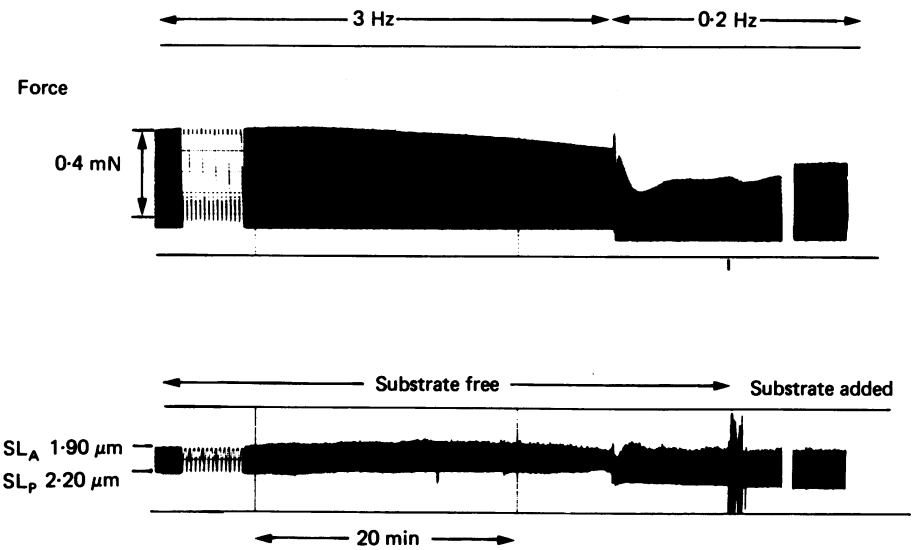

Fig. 6. Isolated rat trabeculae. The effect of substrate withdrawal at a stimulus frequency of 3 Hz. There was no effect on mechanical performance for 10 min but deterioration was apparent after 30 min. SL = sarcomere length; A = active; P = passive.

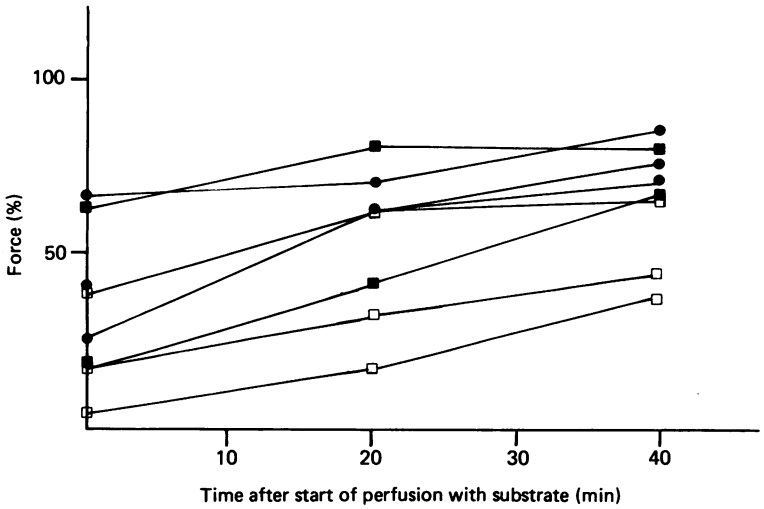

Fig. 7. Isolated rat trabeculae, replacement of exogenous substrate, following depletion of endogenous substrate stores. The recovery rate was the same regardless of whether lactate (open squares), glucose (filled circles) or palmitate (filled squares) was added.

(Henderson, Most & Sonnenblick, 1969; Severeid, Connor & Jong, 1969; Opie, Lochner, Owen, Bruyneel, Whitelaw, Lubbe & Mansford, 1972).

Myocardial oxygen consumption is a function of load and mechanical performance (Weber & Janicki, 1977; Elzinga & Westerhof, 1980). Therefore, in order to study the effect of substrates, these mechanical determinants must be held *constant*. In order to achieve this and to avoid secondary effects, such as catecholamine release, which

may occur in intact animal experiments, we used an isolated heart but allowed it to eject into a normal input impedance and perfuse its own coronary arteries (Elzinga & Westerhof, 1980). As deficient oxygen supply may influence the effect of an increased oxygen demand, we used red blood cells in the perfusion fluid providing a high arterial oxygen content (Table 2). Under these circumstances we find no effect of palmitate on myocardial oxygen consumption or efficiency.

The previous reports of the effects of free fatty acids on myocardial oxygen consumption conflict in two ways: (1) within species and (2) between species. (1) Both an increase and a decrease in myocardial oxygen consumption has been reported in isolated perfused rat Langendorff hearts and both an increase and no effect have been reported in intact anaesthetized dogs (Table 1). (2) Free fatty acids increase myocardial oxygen consumption in swine but not in man (Table 1). Part of the discrepancy between our results and previously reported effects of free fatty acids on oxygen consumption may be due to some secondary effect, e.g. in the paper of Liedtke, Nellis & Neely (1978), increased oxygen consumption with free fatty acids was associated with a higher heart rate, probably due to an adrenergic response.

Our results are in agreement with those of Most, Lipsky, Szydlik & Bruno (1973) in the intact dog, and Rogers, McDaniel, Moraski, Rackley & Russell (1977), in man. These last authors argue that the lack of effect was probably due to free fatty acids being stored as triglycerides rather than oxidized. In order to determine the proportion of increased free fatty acid uptake which was oxidized, we would have required [^{14}C]palmitate as substrate and measurements of $^{14}CO_2$ production (Riemersma, Holland, Owen, Lewis & Opie, 1971/72). However, even this procedure would not give us any assurance about the way the heart would utilize other fatty acids (Van der Vusse & Reneman, 1983). Thus, we have no direct evidence on the fate of the increased palmitate uptake, but the simultaneous fall in lactate consumption (Fig. 5) and glucose consumption argues in favour of increased free fatty acid oxidation. Thus we conclude that in cat, as in man, increased oxidation of free fatty acids (at least when given as palmitate) also has no effect on oxygen consumption.

The only significant effect of palmitate on the mechanical performance of the heart was a slight increase in the maximum rate of rise of left ventricular pressure (dP/dt_{max}). We do not know the reason for this. There were no significant changes in any other variable apart from the transient effect immediately following the change in perfusate (Fig. 2). These findings are contrary to those reported in isolated perfused hearts by Severeid *et al.* (1969) and Henderson *et al.* (1969, 1970*a*, *b*) who found a deleterious effect of free fatty acids on cardiac performance in rat Langendorff hearts. However, our results on mechanical performance agree with authors who studied intact animal preparations (Table 1). Oxygen supply of hearts perfused with fluids containing no haemoglobin may be inadequate; lactate production is indicative of such inadequacy. Even in our preparation, lactate production was found in one heart (Fig. 5, beginning with open squares) but only during the exposure to palmitate. It is possible that in the other preparations, palmitate had some smaller effect on anaerobic metabolism which was not detected. Nevertheless, there was no deterioration in mechanical performance (Figs. 2 and 3, Table 3) even in the heart which did produce lactate.

In the intact dog heart an increase in arterial lactate concentration to above 4·5 mM causes an increase in lactate oxidation to a value of over 80 % of total CO_2 production

(Drake, Haines & Noble, 1980). Willebrands & van der Veen (1967) found that lactate increased oxygen consumption in isolated rat hearts. However, their preparation was anaerobic when perfused with glucose (it produced lactate) and endogenous substrate stores were depleting during the perfusion. Clearly such an investigation depends on the use of a preparation in which aerobic metabolism and therefore lactate oxidation is unimpeded. Our study shows that under these circumstances there is no discernable effect on mechanical performance or oxygen consumption of the cat heart.

A factor which we were unable to assess was the possible utilization in these experiments of endogenous substrates. The effect of a switch to endogenous substrate can be studied by removing exogenous substrate from the perfusion medium. This is not possible to do completely in red cell suspensions because the red cells generate lactate. Removal of the red cells causes tissue hypoxia. To overcome this problem, we turned to isolated superfused tissue with very small dimensions but unfortunately this necessitated a change of species (rat). These trabeculae were sufficiently small to allow full oxygenation and measurements of sarcomere length by laser diffraction but it was not possible to measure oxygen consumption; only mechanical performance was measured. The results suggest that utilization of endogenous substrate has no effect on mechanical performance until those substrates become depleted. It therefore seems very unlikely that our conclusions concerning the cat heart experiments are affected by the possibility of some endogenous store utilization. These experiments on trabeculae also confirm the lack of effect of different exogenous substrates on average mechanical performance during the control period, and on rate of recovery following endogenous substrate store depletion.

The calorific equivalent of oxygen was assumed to be constant (4·8 kcal per l.) throughout these experiments. However a switch from 100 % carbohydrate to 100 % lipid oxidation involves a change of calorific equivalent of oxygen from 5·047 to 4·686 (kcal per l.). This would be the source of an error in our efficiency calculations if the same factors that apply in calorimetry also pertain to myocardial oxidation. However, a further complication is that glucose (but not lactate) oxidation yields two extra ATPs on anaerobic conversion to pyruvate. Furthermore, the switch to lipid oxidation in the palmitate experiments is not 100 %. As already stated in this paper, we were unable to determine the proportional change in the absence of a [^{14}C]palmitate label. Taking the extreme case of a 10 % decrease in calorific equivalent (5·047 to 4·686 kcal per l.), the efficiencies in series B, Table 3, would be 23·2 % for the control period and 25·4 % for the palmitate period. Although this is a statistically significant change ($P = 0·05$), this conclusion, that fat increases the efficiency of the heart, is opposite to that stated in the literature and is heavily dependent on the assumptions made.

Mechanical efficiency can be influenced by a change in the proportion of energy channelled to contractile effort as opposed to other intracellular processes. In a previous study in the dog (Gibbs, Papadoyannis, Drake & Noble, 1980) this proportion was found to be about 60 % of total. Therefore, if we had found a change in efficiency, it would have been interesting to repeat that study with different substrates. However, we know of no evidence that ATP, produced from different substrate oxidative processes, is used preferentially by particular ATP consuming processes.

We have not tested the other exogenous substrates (such as pyruvate and ketone

bodies) which do not achieve high arterial concentrations under physiological circumstances. However, the three substrates we have used enter the oxygen consuming, energy producing, tricarboxylic acid cycle, through different representative routes. These routes are shared by endogenous carbohydrate and lipid substrates. Therefore, our over-all conclusion is that the fully aerobic heart consumes whatever substrate is available without any change in efficiency.

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