Lipid accumulation in isolated perfused rat hearts has no apparent effect on mechanical function or energy metabolism as measured by ^{31}P NMR

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Abstract Male Sprague-Dawley rats were fed diets that contained 20% by weight soybean oil or rapeseed oil (21% and 43% erucic acid) for 7 days. The rapeseed oil diets increased the cardiac triacylglycerol content 5-fold and 25-fold, respectively, above control values. Hearts were removed from the animals and perfused with modified Krebs-Henseleit buffer at 37°C. The calculated rate-pressure product was used as a measure of contractile function. ^{31}P NMR spectra were acquired throughout a protocol that consisted of 12 min control perfusion, followed by 12 min perfusion with 20 μM isoproterenol, 12 min washout, 12 min total global ischemia, and 28 min reperfusion. The steady state levels of creatine phosphate, ATP, intracellular pH, contractile function, and the free energy of ATP hydrolysis (ΔG_{ATP}) were determined for all three groups of hearts. Isoproterenol more than doubled the rate-pressure product of the hearts on all diets and decreased the concentrations of creatine phosphate and ATP with a concomitant rise in Pi. After global ischemia, creatine phosphate levels recovered fully, ATP levels remained low, and most hearts developed ventricular fibrillation. Changes in intracellular pH were the same for all groups: pH was 7.1 throughout the equilibration and isoproterenol perfusion period, decreased to pH ~6.4 during ischemia, and returned to 7.0 during reperfusion. The results indicate that the fat accumulation that occurs in the hearts of rats fed diets rich in high erucic acid rapeseed oil does not interfere with the cardiac high energy phosphate metabolism or contractile function. These data imply that the free fatty acid in these hearts did not reach the levels required to uncouple oxidative phosphorylation.—**Stewart, L. C., J. K. G. Kramer, F. D. Sauer, K. Clarke, and M. S. Wolynetz.** Lipid accumulation in isolated perfused rat hearts has no apparent effect on mechanical function or energy metabolism as measured by ^{31}P NMR. *J. Lipid Res.* 1993. **34:** 1573–1581.

Supplementary key words ATP • energy metabolism • erucic acid • triacylglyceride accumulation

Dietary C_{22} monounsaturated fatty acids, such as erucic (EA, 22:1n-9) and cetoleic (22:1n-11) acids found in high erucic acid rapeseed (HEAR) oils and some fish oils, respectively, have been shown to cause accumulation of triacylglycerol (TAG) in rat hearts (1–3). The buildup of TAG was proportional to the dietary concentration of these fatty acids. On continuous feeding, a maximum was reached after 3–7 days, and declined thereafter presumably due to increased peroxisomal β-oxidation (3). In the rat heart, the incorporation of EA was highest in TAG, with intermediate levels found in sphingomyelin (SP), phosphatidylserine (PS), and diphosphatidylglycerol (DPG), and small amounts in the remaining phospholipids (4–7). The maximum level of EA incorporated into the cardiac phospholipids was attained after feeding rats HEAR oils for 1 week (7).

The earlier studies on oxidative phosphorylation in rat hearts were conducted with isolated mitochondria. It was reported that rat hearts at maximum TAG and EA accumulation showed a 70% reduction in ATP synthesis and up to 60% inhibition of the oxidation of some substrates (8). In addition, EA inhibits the oxidation of palmitic acid and other fatty acids (9). Several investigators confirmed reduced ATP synthesis (6, 10–12) and impaired oxidation of some fatty acids (6, 10–13) in heart mitochondria of rats fed HEAR oils. It is largely for this reason that EA is still referred to as "toxic" (14–16). However, whether the EA-induced changes in TAG content and EA incorporation affects mitochondrial function remains controversial (17–20).

Abbreviations: CrP, creatine phosphate; DPG, diphosphatidylglycerol; EA, erucic acid; FFA, free fatty acids; ΔG_{ATP}, free energy of ATP hydrolysis; GC, gas chromatography; HEAR, high erucic acid rapeseed; HPLC, high pressure liquid chromatography; NMR, nuclear magnetic resonance; PC, phosphatidylcholine; PE, phosphatidylethanolamine; Pi, inorganic phosphate; PI, phosphatidylinositol; PS, phosphatidylserine; RPP, rate-pressure product; SBO, soybean oil; SP, sphingomyelin; TLC, thin-layer chromatography; TAG, triacylglycerol.

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The results with isolated heart mitochondria have been questioned (21) after it was demonstrated that in perfused hearts fatty acid oxidation rates were not significantly different between rats fed HEAR oil or any other vegetable oil (22, 23) and peroxisomal β-oxidation was involved in the metabolism of EA (3). However, to date no direct measurements on the intact heart have been made to definitively answer the question whether ATP production rates and oxidative capacity are reduced in hearts of rats at maximum load of TAG and EA.

In the present study, we used ^{31}P NMR on intact perfused rat hearts during elevated TAG and EA accumulation to measure high energy phosphates on a continuous basis, in order to avoid possible artificial results associated with the isolation procedures of heart mitochondria. During spectral acquisition, mechanical function and coronary flow were measured. The results were compared to those from hearts of rats fed soybean oil (SBO) at the same level of fat in the diet. The ability of isolated hearts to respond to increased work load, ischemia, and reperfusion was assessed by ^{31}P NMR spectroscopy. ^{31}P NMR is particularly suited for such studies since it is noninvasive. The method was used recently by other investigators to demonstrate uncoupling of oxidative phosphorylation by free fatty acids (FFA) (24).

MATERIALS AND METHODS

Animals and diets

Male Sprague-Dawley rats (n = 45) weighing approximately 360 g were fed semisynthetic diets (25) for 7 days containing the following test oils at 20% by weight of the diet: soybean oil (SBO), a mixture of high erucic acid rapeseed oil and canola oil (21% EA), and high erucic acid rapeseed oil (43% EA). The fatty acid composition of the dietary oils are shown in **Table 1.**

TABLE 1. Fatty acid composition of dietary oils

Fatty Acids	Soybean Oil	HEAR Oil (21% EA)[a]	HEAR Oil (43% EA)
		weight %	
14:0	0.1	0.1	0.1
16:0	9.9	4.8	3.2
16:1	0.2	0.3	0.2
18:0	3.6	2.0	1.2
18:1n-9	23.8	35.2	19.0
18:1n-7	1.5	2.3	0.8
18:2n-6	50.0	19.3	14.3
18:3n-3	8.7	8.9	8.4
20:0	0.4	0.9	0.8
20:1n-9	0.4	3.6	6.3
22:0	0.4	0.7	0.7
22:1n-9	0.0	20.7	42.9
24:0	0.1	0.3	0.3
24:1n-9	0.0	0.7	1.1

HEAR, high erucic acid rapeseed; EA, erucic acid, 22:1n-9.
[a]HEAR oil containing 21% EA was prepared by mixing canola oil (0.8% EA) and HEAR oil (42.9% EA).

Heart perfusion

Rats were anesthetized by intraperitoneal injection of sodium pentobarbital. The hearts were quickly removed and perfused via the coronary arteries at a constant pressure of 100 mm Hg and at 37°C. Following placement of a left ventricular apical drain, a water-filled balloon was inserted through the mitral valve and secured in the left ventricle. The balloon was connected to a Statham DB23 pressure transducer and a Gould 2-channel physiological recorder for monitoring the heart rate and ventricular pressures. The rate-pressure product (RPP), the product of heart rate and developed pressure (systolic minus diastolic pressures) was used as an index of contractile function. Coronary flow was measured throughout the protocol.

Hearts were perfused with a modified Krebs-Henseleit buffer containing 118 mM NaCl, 25 mM NaH_2CO_3, 4.7 mM KCl, 1.75 mM $CaCl_2$, 1.2 mM $MgSO_4$, 0.5 mM EDTA, and 11 mM glucose. The perfusate was equilibrated with 95% O_2/5% CO_2, maintaining a pH of 7.4.

The experimental protocol was as follows: 20 min equilibration prior to NMR spectral acquisition, 12 min control perfusion (with acquisition of NMR spectra), 12 min perfusion with 20 μM isoproterenol, 12 min washout of isoproterenol, 12 min total global ischemia, and 28 min reperfusion. All hearts were freeze-clamped at the end of the protocol for lipid analyses. Hearts from other rats within each diet group (n = 25) were isolated and perfused for 20 min, then freeze-clamped and analyzed to provide control biochemical and lipid data.

Isoproterenol was delivered to the hearts from an infusion pump through a separate line into the buffer directly above the aortic cannula at a rate of < 1% of coronary flow for each heart. Total global ischemia was induced by clamping the perfusion line to the cannula. Temperature was maintained at 37°C throughout the experimental protocol.

Phosphorus NMR spectroscopy

^{31}P NMR spectra were acquired using a Bruker wide-bore AM 360 spectrometer operating at 145.75 MHz. Spectra were acquired with 60° pulses (24 μs) and an interpulse delay of 2.3 s (104 accumulations; 4 min time resolution). The sweep width was ± 12 KHz and 8 K data points were collected.

Calculations

^{31}P NMR resonance areas were calculated using the NMR1 program (NMRi, Syracuse, NY) on a SUN 3 data station. Exponential multiplication of the free induction decay was used for sensitivity enhancement (20 Hz line broadening). A representative spectrum is shown in **Fig. 1.** ^{31}P NMR peak areas were corrected for partial saturation (Pi, 15%; CrP, 20%; ATP, 10%). For each group of

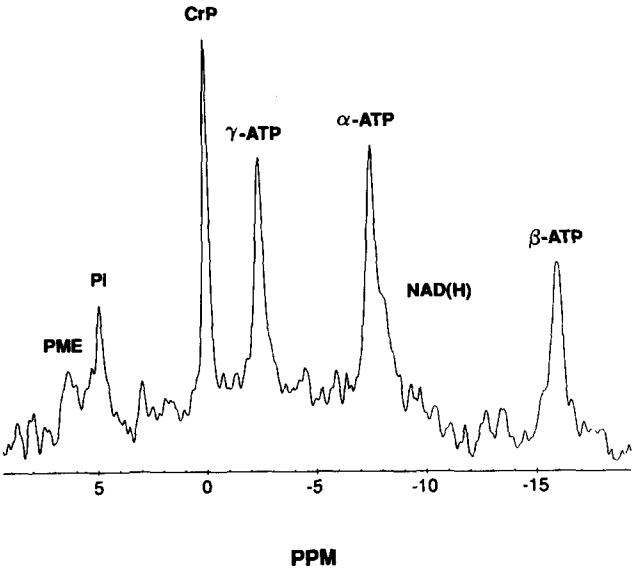

Fig. 1. Representative ^{31}P NMR spectrum obtained from an isolated rat heart (SBO group) showing peaks for ATP (α, β, and γ-P), NAD(H), creatine phosphate (CrP), inorganic phosphate (Pi), and phosphomonoesters (PME).

hearts, the averages of the ATP concentrations for the first three spectra (control period) were set to ATP values determined by high pressure liquid chromatography (HPLC) for each experimental group. Intracellular pH was calculated from the chemical shift of the Pi resonance relative to phosphocreatine (26) using a standard curve of the chemical shift of Pi versus pH.

Since free ADP is present in concentrations too low to be measured directly from the ^{31}P NMR spectra, the concentration was calculated from the creatine kinase reaction which is assumed to be close to equilibrium at all times.

$$[ADP] = [ATP][Cr]/[CrP][H^+]K_{CK}$$

where K_{CK} was 1.66×10^9 mol^{-1} (27). The free-energy change of ATP hydrolysis (ΔG_{ATP}) was calculated from the equation:

$$\Delta G_{ATP} = \Delta G°_{obs} + RT \ln \frac{[ADP][Pi]}{[ATP]}$$

At 37°C and a magnesium concentration of 1 mM, the standard free-energy change ($\Delta G°_{obs}$) was -30.5 kJ/mol (28). The gas constant (R) was 8.31 J/deg/mol and the absolute temperature (T) was 310°K. The values of both K_{CK} and $\Delta G°_{obs}$ are known to vary with changes in temperature, pH and $[Mg^{2+}]$ (28). Changes in the values of K_{CK} and ΔG due to changes in pH seen in these protocols resulted in changes in ΔG_{ATP} of $< 10\%$ (27, 29). Temperature was maintained at 37°C throughout the experimental protocol, and changes in $[Mg^{2+}]$ during ischemia have been shown to be too small to lead to significant errors in $\Delta G°_{obs}$ or K_{CK} (30).

Biochemical and lipid analyses

ATP (31), lactate, glycogen (32), wet/dry weight ratios, protein (33) assays, and lipid analyses were performed on a separate series of hearts, freeze-clamped at the end of the 20-min equilibration period, and stored in liquid N_2. Hearts at the end of the protocol were also freeze-clamped.

Total lipids were obtained from a portion of the heart after pulverization at dry ice temperature and extraction with chloroform–methanol 1:1 (34). Lipid classes were separated by 3-directional TLC (35) and quantitated by GC (36) after addition of methyl heptadecanoate as internal standard (37) and transesterification using anhydrous 5% HCl–methanol (w/w).

Statistics

The biochemical and lipid data were examined using a one-way ANOVA. To examine dietary differences, the NMR data from each stage of the protocol were analyzed separately using a one-way ANOVA. To compare protocol differences, the data were analyzed as a split-plot in time. This permitted protocol differences to be examined on a within-heart basis.

RESULTS

The average weight gain after 1 week on the diets was 58 ± 4 g and body weights were 410 ± 14 g. All analyses are reported for the heart after 20 min of perfusion with Krebs-Henseleit buffer containing 11 mM glucose (control perfusion). The values are expressed as mg/g protein because cardiac TAG in HEAR oil-fed rats significantly increased the dry weight content of the heart.

Control perfusion

Cardiac lipids. Rats fed the two HEAR oil diets for 1 week showed the characteristic buildup of cardiac TAG (9.6, 54.5, and 288 mg/g protein for SBO, 21% EA, and 43% EA, respectively). The differences in the concentration of EA per g protein between the two HEAR oil diets was 7.2-fold greater based on the cardiac TAG level alone, and 6.5-fold greater based on all cardiac lipids. By the end of the experimental protocol, the content of cardiac TAG had decreased significantly in both the SBO (3.8 mg/g protein) and 21% EA (14.4 mg/g protein), while in the 43% EA diet the cardiac TAG level remained high (443 mg/g protein). A slight decrease of EA was evident in cardiac TAG on extended perfusion with both HEAR oil diets (28.8 to 23.1% for diet 43% EA; 21.2 to 18.8% for diet 21% EA).

The level of cardiac free fatty acids (FFA) during control perfusion was low for all diets, and did not change

TABLE 2. Fatty acid composition (in weight %) of selected fatty acids in the cardiac lipids of rat hearts perfused for 20 min (control perfusion)

Fatty Acid	Lipid Class[a]	Diets[b] SBO (n = 4)	21% EA (n = 4)	43% EA (n = 5)	SEM[c]	Fatty Acid	Diets SBO (n = 4)	21% EA (n = 4)	43% EA (n = 5)	SEM
22:1n-9	TAG	tr[1][d]	21.2[2]	28.8[3]	1.5	18:2n-6	31.9[1]	13.3[2]	12.0[2]	0.7
	FFA	tr[1]	2.5[2]	6.1[3]	2.4		5.5	2.7	3.1	1.0
	PC	0[1]	1.1[2]	3.1[3]	0.2		16.5	12.1	13.4	2.4
	PE	0[1]	1.1[2]	2.9[3]	0.2		10.8[1]	7.5[1,2]	6.8[2]	1.1
	DPG	0[1]	0.6[2]	4.5[3]	1.0		88.7[1]	86.0[1]	70.1[2]	3.1
	PS	tr[1]	5.2[2]	6.4[3]	0.4		3.5	2.8	3.6	0.4
	PI	0[1]	0.4[2]	0.8[2]	0.1		9.5[1]	5.3[2]	4.5[2]	0.5
	SP	0.1[1]	1.8[2]	4.4[3]	0.6		4.0	1.4	0.8	1.1
20:4n-6	TAG	2.2[1]	1.0[2]	0.7[2]	0.5	Σ C22	1.6	1.5	1.4	0.4
	FFA	2.7	2.1	1.8	1.1	PUFA	1.6	1.1	0.6	0.3
	PC	20.4	23.7	20.2	3.3		5.5	4.0	4.0	0.9
	PE	19.0[3]	22.1[1]	20.6[2]	0.4		20.7	24.4	20.7	2.2
	DPG	1.0[2]	1.0[2]	1.6[1]	0.1		1.2	1.4	2.1	0.4
	PS	7.0[1]	5.5[1,2]	4.1[2]	0.5		18.0	13.9	16.5	2.1
	PI	17.9	21.4	21.2	1.2		1.9	2.2	2.6	0.5
	SP	1.0	2.4	0.5	0.7		0.5	0.3	0.3	0.1

[a]For abbreviations of lipid classes see Abbreviations; PUFA, polyunsaturated fatty acids; tr, trace.
[b]SBO, soybean oil; 21% EA and 43% EA, high erucic acid rapeseed oils containing 21 or 43% erucic acid (EA). All diets contained oil at 20% by weight.
[c]SEM, pooled standard error of the mean.
[d]Means with different superscript numbers are significantly different ($P < 0.05$).

throughout the perfusion protocol from 20 min to the end of the protocol (0.9, 1.5, and 0.9 mg/g protein for SBO, 21% EA, and 43% EA, respectively). The EA content of cardiac FFA after 20 min perfusion is shown in **Table 2.** The level of EA increased significantly by the end of the perfusion protocol to about 10% for both HEAR oil diets. There were no significant differences between diets and time of perfusion in the content of cardiac cholesterol (~7.5 mg/g protein) or cholesterol esters (~0.4 mg/g protein).

There were no significant differences in cardiac phospholipids of rats fed SBO or HEAR oil with 21% EA, but the feeding of the HEAR oil diet with 43% EA resulted in a significant decrease of some phospholipids compared to SBO (PC, 59 vs. 52; PE, 38 vs. 30; DPG, 18 vs. 15; alkenylacyl PE, 1.8 vs. 0.7 mg/g protein), and an increase in PI (2.6 vs. 5.4 mg/g). The relative concentration of EA was highest in PS followed by SP and DPG, and lowest in PI (Table 2). The relative concentration of EA did not change on extended perfusion in any of the phospholipids in either HEAR oil diet (data not shown).

Biochemical analyses. Tissue ATP, lactate, and glycogen levels, after 20 min of perfusion, are summarized in **Table 3.** Statistical analyses showed that HPLC-derived ATP values were significantly lower for the 43% EA group compared to SBO, while the 21% EA group was not significantly different. There were no significant differences in ADP values (137, 138, and 170 ± 28 nmol/g protein for SBO, 21% EA, and 43% EA, respectively) and the ATP/ADP ratio (261, 228, and 158 ± 48 for SBO, 21% EA, and 43% EA, respectively). ATP levels determined by HPLC at the end of the experimental protocol were low (results not shown), consistent with the NMR results and previous studies which have shown that purines are lost from the myocardium during reperfusion (38, 39). The differences in tissue glycogen levels at the beginning of the protocol were not significant ($P > 0.05$) for the three dietary groups (Table 3). Glycogen levels at the end

TABLE 3. ATP, lactate, and glycogen content in rat hearts after 20 min perfusion determined biochemically

Diets[a]	n	ATP	Lactate	Glycogen[b]
		μmol/g protein		
SBO	5	35.7[1,c]	8.3	120.7
21% EA	3	31.4[1,2]	6.6	98.9
43% EA	4	26.8[2]	12.2	99.4
SEM[d]		2.0	3.2	20.1
ANOVA[e]		*	NS	NS

[a]SBO, soybean oil; 21% EA and 43% EA, high erucic acid rapeseed oils containing 21 or 43% erucic acid (EA). All diets contained oil at 20% by weight. For the three diets, respectively: dry wt % of wet wt: 14 ± 1; 14 ± 2; 16 ± 2; protein (mg/g dry wt): 657 ± 21; 599 ± 7; 573 ± 23.
[b]Glycogen was expressed as glucose equivalents.
[c]Within a column, means with different superscript numbers are significantly different ($P < 0.05$).
[d]Pooled standard error of the mean (SEM) based on n = 3.
[e]Effect of diet: NS, not significant ($P < 0.05$); *, $P < 0.05$.

of the experimental protocol were at the limit of detection by this assay (2-4 mg glucose equivalents/g wet weight) and again were not significantly different between diets. Lactate levels during control perfusion were low in all hearts, and remained low throughout the perfusion protocol as is expected with well oxygenated preparations.

Perfusion results. Mechanical function, as assessed by RPP, was not significantly different among diets (28557, 25741, and 30526 ± 3130 mm Hg/min for SBO, 21% EA, and 43% EA, respectively). Coronary flows were comparable for all groups of hearts during control perfusion at about 25 ml/min.

Ratios of CrP/ATP (1.5-1.7) were not significantly different among diets during control perfusion (**Table 4**). In addition, Pi was lower during control perfusion in the HEAR oil-fed rat hearts. The combination of the higher

TABLE 4. Inorganic phosphate (Pi), creatine phosphate (CrP), ATP, and pH values determined from NMR spectra of perfused hearts of rats throughout the experimental protocol

Protocol[a] (No. of NMR/ protocol)	Diet[b] SBO (n = 4)[e]	21% EA (n = 4)[e]	43% EA (n = 5)[e]	SEM[c]	Diet Differences[d]
		μmol/g protein			
Pi					
Control (3)	23.3[1][f]	12.5[2]	12.0[2]	2.6	*
Isoproterenol[g] (3)	44.3[1]	31.7[1,2]	24.8[2]	4.6	*
Washout (3)	14.1	11.1	13.7	3.4	NS
0-4 min Ischemia (1)	82.5[1]	61.4[2]	57.4[2]	5.1	**
5-8 min Ischemia (1)	102.9[1]	85.0[2]	75.6[2]	5.1	**
9-12 min Ischemia (1)	139.2[1]	105.2[2]	93.6[2]	5.1	**
Reflow (7)	58.8[1]	36.0[2]	36.0[2]	6.0	*
SEM[h]		3.9 ****			
CrP					
Control	54.0	53.3	46.3	3.7	NS
Isoproterenol	41.7	38.9	37.0	3.0	NS
Washout	65.6[1]	59.7[1,2]	51.3[2]	3.4	*
0-4 min Ischemia	12.7	13.7	11.4	1.8	NS
5-8 min Ischemia	3.5	2.5	0		
9-12 min Ischemia	0	4.1	0		
Reflow	52.0[1]	47.0[1,2]	35.6[2]	4.7	(P = 0.06)
SEM		0.9 ****			
ATP					
Control	35.7[1]	31.3[1,2]	27.0[2]	1.5	**
Isoproterenol	28.6[1]	25.2[1,2]	20.8[2]	2.2	(P = 0.07)
Washout	28.1[1]	23.2[2]	19.6[2]	1.4	**
0-4 min Ischemia	19.5	18.9	15.1	1.8	NS
5-8 min Ischemia	10.2	11.6	8.8	1.8	NS
9-12 min Ischemia	4.2	7.0	3.8	1.8	NS
Reflow	7.8	9.7	5.5	2.8	NS
SEM		0.8 ****			
pH					
Control	7.1	7.1	7.1	0.02	NS
Isoproterenol	7.1	7.1	7.1	0.01	NS
Washout	7.1	7.1	7.1	0.04	NS
0-4 min Ischemia	6.8	6.8	6.7	0.03	NS
5-8 min Ischemia	6.5	6.5	6.5	0.03	NS
9-12 min Ischemia	6.4	6.4	6.4	0.03	NS
Reflow	7.0[1]	7.0[1]	6.9[2]	0.02	*
SEM		0.01 ****			

[a] Within each experimental protocol there are a number of 4-min accumulations of NMR signals given in brackets.
[b] SBO, soybean oil; 21% EA and 43% EA, high erucic acid rapeseed oils containing 21 or 43% erucic acid (EA). All diets contain oil at 20% by weight.
[c] Pooled standard error of the mean (SEM) for comparing diet means, based on n = 4.
[d] Differences are indicated. NS, not significant, *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.
[e] Number of rat hearts examined per diet.
[f] Within a protocol, means having different superscript number are significantly different ($P < 0.05$).
[g] Perfusate contained 20 μM isoproterenol.
[h] Pooled SEM to compare the within-diet changes between control, isoproterenol, washout, ischemia, and reflow means, based on n = 4 rats/diet/protocol. The patterns of change for the three diets for Pi ($P = 0.06$), CrP ($P = 0.09$), ATP (NS), and pH (NS) were not significant.

ratio of CrP/ATP and the lower Pi was not reflected in the ΔG_{ATP} values in hearts of rats fed either HEAR oil or SBO (−58.5, −59.2, and −58.8 ± 0.8 kJ/mol for SBO, 21% EA, and 43% EA, respectively).

Isoproterenol infusion

Infusion with 20 μM isoproterenol for 12 min caused a ≥ 2-fold increase in RPP (60392, 56109, and 60917 ± 5083 mm Hg/min for SBO, 21% EA, and 43% EA, respectively) and a concomitant significant decrease in both ATP and CrP and a significant increase in Pi (Table 4). The ratio of CrP/ATP remained ~1.5 for SBO and 21% EA, but increased significantly to 1.8 for the 43% EA group. There were no changes in pH in all groups (Table 4). The increased RPP during isoproterenol infusion significantly decreased ΔG_{ATP} values by 2–4 kJ/mol compared to the corresponding control values, but there were no significant diet differences (−55.1, −55.3, and −56.1 ± 0.7 kJ/mol for SBO, 21% EA, and 43% EA, respectively). Prior to ischemia, the hearts were re-equilibrated with buffer in the absence of isoproterenol, to allow the function to return to the pre-isoproterenol levels. Pi and ΔG_{ATP} (−61.5, −60.5, and −58.3 ± 0.9 kJ/mol for SBO, 21% EA, and 43% EA, respectively) recovered, CrP increased, and ATP and RPP (20757, 16847, and 21982 ± 2817 mm Hg/min for SBO, 21% EA, and 43% EA, respectively) decreased significantly. The CrP/ATP ratio increased significantly in all diets to ~2.5, but there were no diet differences. The pH values remained unchanged.

Coronary flows increased 1.2- to 1.5-fold during isoproterenol infusion and returned to control values during washout.

Ischemia and reperfusion

During ischemia, heart rate and developed pressure decreased to zero within 3–5 min for all hearts. During reperfusion, none of the SBO hearts recovered function, but two hearts on each of the 21% and 43% EA diets recovered significant function. The remaining hearts developed ventricular fibrillation.

ATP and CrP decreased to low levels within 8 min of ischemia on all diets (Table 4) with a concomitant significant increase in Pi and a significant decrease in pH. Both the increase in Pi and the decrease in pH were significantly lower for the 43% EA diet. During reperfusion, CrP and pH generally recovered to pre-ischemic values, but there was little recovery of ATP, probably because the hydrolysis products of ATP (primarily adenosine and inosine) are lost during reperfusion (38, 39). Pi values remained significantly higher than control values even after 28 min of reperfusion. During reperfusion, ΔG_{ATP} partially recovered (−56.3, −56.3, and −55.1 ± 0.8 kJ/mol for SBO, 21% EA, and 43% EA, respectively).

The pattern of change within each of the three diets was not significantly different throughout the protocol (Table 4), i.e., results expressed relative to control values (average of first 12 min of perfusion) were not significantly different between diets (**Fig. 2**).

DISCUSSION

The feeding of HEAR oil to rats for 1 week resulted in elevated accumulation of cardiac TAG, as well as incorporation of EA into cardiac lipids. These changes in lipid content and fatty acid composition were suspected of interfering with energy production in the heart. Decreased ATP production in isolated rat heart mitochondria (6, 8, 10–12) and impaired oxidation of palmitic acid and other fatty acids (6, 9–13) was provided as evidence. However, several investigators could not confirm decreased ATP production (17, 19) and impaired fatty acid oxidation (17–20) in isolated rat heart mitochondria.

The abnormalities of EA were at first attributed to the presence of high levels of FFA (8). However, these high levels of cardiac FFA in rats fed HEAR oils were subsequently shown to be an artifact of inappropriate extraction procedures (34). In the present study, the level of FFA in perfused hearts was shown to be about 1 mg/g protein (or 80 μ/g wet wt), similar to that reported previously in non-perfused hearts of rats fed HEAR oil (34, 40).

There was extensive accumulation of EA-rich cardiac TAG and phospholipids containing appreciable amounts of EA in rats fed HEAR oil for 1 week. The levels were similar to those observed after long-term feeding of HEAR oil (7). Furthermore, there was a increase in the C_{22}n−3 polyunsaturated fatty acids derived from linolenic acid in the dietary oils. However, despite the extensive changes in membrane lipids (4, 41, 42), the overall work function was not impaired.

Normally, there is a precise balance between myocardial energy, supply and demand. A decreased rate of ATP synthesis through uncoupling of oxidative phosphorylation, for example, should therefore lead to a decrease in either steady state [ATP] and [CrP] and/or to a decrease in contractile function. It has been shown that there is a significant increase in O_2 consumption and a decrease in rate of ATP synthesis and in RPP in rat hearts uncoupled with 2,4-dinitrophenol or FFA (24). However, the buffer FFA levels required to produce uncoupling were high (6 mM, or 13 mg/g protein).

To test whether the presence of elevated myocardial levels of TAG, and EA-enriched TAG and phospholipids, leads to abnormalities in myocardial energy production, we compared mechanical function (RPP) and energetic state ([ATP], [CrP], ΔG_{ATP}) in buffer-perfused hearts isolated from rats fed 20% by weight SBO, 21% EA HEAR oil, or 43% EA HEAR oil. In addition to the period of control perfusion, hearts were challenged with isoproterenol infusion, followed by ischemia and reperfusion. Perfusion

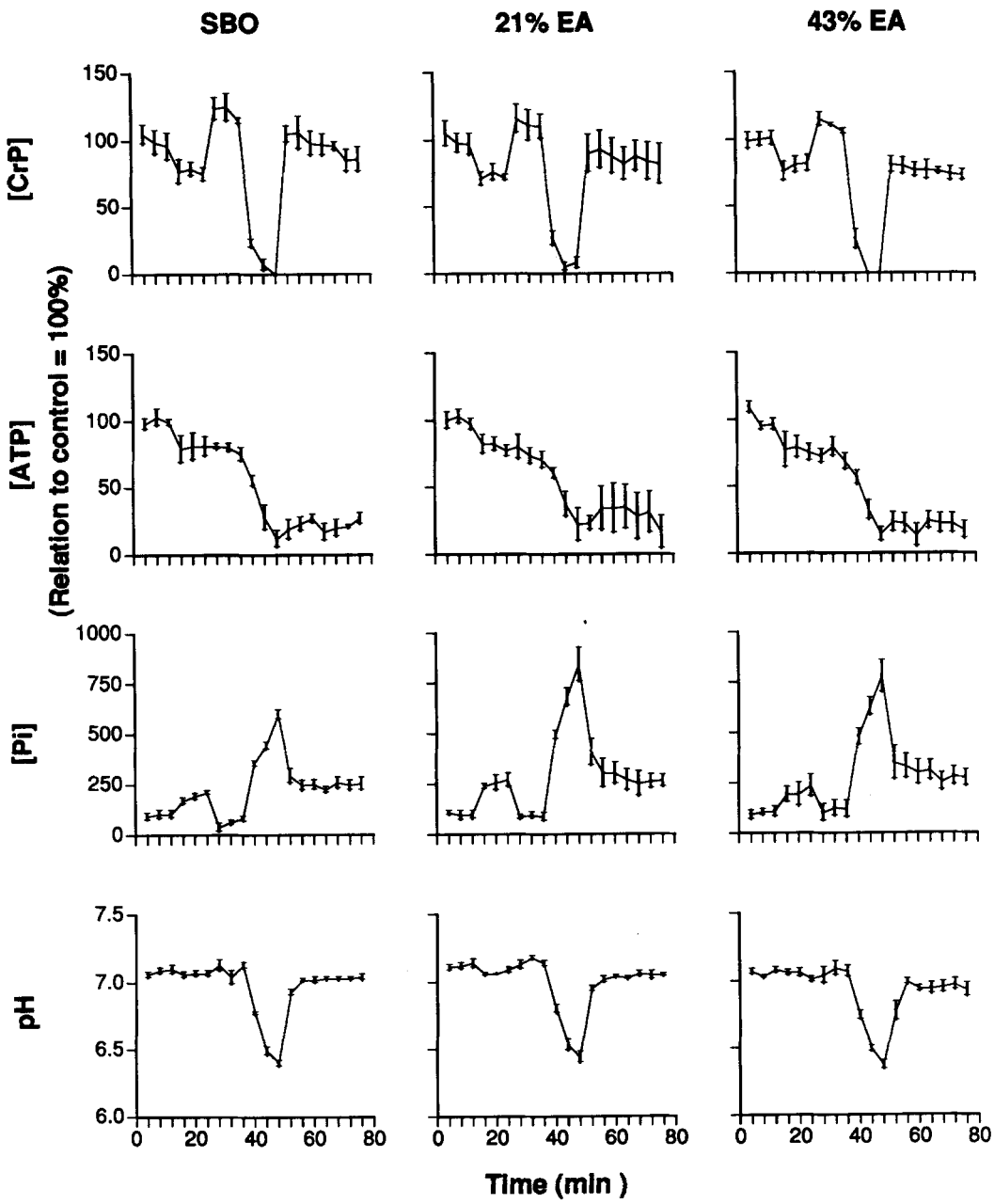

Fig. 2. Time course of changes in CrP, ATP, Pi, and pH in response to isoproterenol, ischemia, and reperfusion for hearts of rats fed SBO, 21% EA (erucic acid) rapeseed oil, and 43% EA rapeseed oil. Points represent the mean ± SD. Control perfusion (0–12 min), isoproterenol (12–24 min), washout (24–36 min), ischemia (36–48 min), and reperfusion (48–76 min). The averages of the three control perfusion values are set to 100% for CrP, ATP, and Pi.

with isoproterenol led to a dose-dependent increase in contractile function secondary to an increase in cytosolic calcium. Ischemia results in well-characterized decreases in levels of high energy phosphate, pH, and mechanical function, due to inhibition of oxidative phosphorylation resulting from limited O_2 availability. In this way, the energy status and contractile function of all groups could be compared at a number of different work loads.

The cardiac ATP content, based on protein concentration, was significantly lower in the 43% EA group compared with the control group (SBO). However, this is not a consistent finding, since other workers did not find decreased cardiac ATP levels under similar conditions with rats fed HEAR oils (19). In any case, recent results suggest that cardiac work output, expressed as peak ventricular pressure or RPP, is correlated to the free energy of ATP hydrolysis (ΔG_{ATP}) but not to the absolute amounts of ATP in heart muscle (43). Specifically, it was noted that a 50% drop in ATP concentration per g of muscle did not result in decreased cardiac work output,

while a decrease in ΔG_{ATP} from −60 kJ/mol to −50 kJ/mol lowered peak ventricular systolic pressure to less than half (43). In the present study, there were no diet-related changes in the important parameters of cardiac function, RPP, ΔG_{ATP}, or response to and recovery from superimposed stress (isoproterenol infusion) and ischemia. Thus, while the 43% EA group had decreased cardiac ATP levels at the start of the perfusion, subsequent responses to isoproterenol infusion, ischemia, and reflow within each diet group were not statistically different (Table 4). This, together with the normal values obtained for RPP and ΔG_{ATP}, suggests that EA-related lipid changes do not impair bioenergetics or work output.

In the present study, failure of most rat hearts to regain full recovery after 12 min of ischemia probably is related to metabolic changes resulting from isoproterenol infusion. For example, we observed that after isoproterenol perfusion, hearts from SBO-fed rats failed to recover after only 8 min of ischemia. Previous results from this laboratory showed, in agreement with Humphrey, Holliss, and Seelye (38) that, without isoproterenol infusion, rat hearts fully recovered even with periods of ischemia lasting up to 28 min. Possibly, the combined effects of ischemia and isoproterenol infusion results in myocardial cation imbalances. It is of interest to note that in this study the ΔG_{ATP} values decreased from about −58 kJ/mol during the control period to about −51 kJ/mol after isoproterenol infusion and ischemia. According to Kammermeier (43), this would be below the energy level required for Ca^{2+} pumping by the sacroplasmic reticulum (−52 kJ/mol of ATP under equilibrium condition and higher with net transfer of Ca^{2+}).

In summary, the results of the present investigation contradict the assertion that elevated myocardial TAG and EA levels in cardiac lipids lead to significant decreases in the energy status or mechanical function in response to metabolic stress relative to control hearts. Our data show that there were no significant differences in work output (RPP) in hearts from rats fed different oils, including HEAR oil, during control perfusion or in response to isoproterenol infusion. The normal ability of these fat-loaded hearts to restore CrP levels after prolonged ischemia would appear to preclude any degree of uncoupling of oxidative phosphorylation or impairment in the rate of ATP synthesis. The FFA concentrations in hearts of rats fed HEAR oil were reported to be twice that found with control oils (40); however, in no case does the FFA concentration exceed 1.5 mg/g protein (or 1 mM) provided that lipolysis is avoided during cardiac FFA extraction (34). This is well below the concentration of 6 mM FFA required for uncoupling of oxidative phosphorylation in rat hearts (24) and supports the results of this investigation, which indicates that the bioenergetics of these hearts are not altered despite the extensive loading of these hearts with TAG and EA.

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