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High intake of saturated fat, but not polyunsaturated fat, improves survival in heart failure despite persistent mitochondrial defects

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Aims The impact of a high-fat diet on the failing heart is unclear, and the differences between polyunsaturated fatty acids (PUFA) and saturated fat have not been assessed. Here, we compared a standard low-fat diet to high-fat diets enriched with either saturated fat (palmitate and stearate) or PUFA (linoleic and α-linolenic acids) in hamsters with genetic cardiomyopathy.

Methods and results Male δ-sarcoglycan null Bio TO2 hamsters were fed a standard low-fat diet (12% energy from fat), or high-fat diets (45% fat) comprised of either saturated fat or PUFA. The median survival was increased by the high saturated fat diet ($P < 0.01$; 278 days with standard diet and 361 days with high saturated fat)), but not with high PUFA (260 days) ($n = 30–35$/group). Body mass was modestly elevated (~10%) in both high fat groups. Subgroups evaluated after 24 weeks had similar left ventricular chamber size, function, and mass. Mitochondrial oxidative enzyme activity and the yield of interfibrillar mitochondria (IFM) were decreased to a similar extent in all TO2 groups compared with normal F1B hamsters. Ca^{2+}-induced mitochondrial permeability transition pore opening was enhanced in IFM in all TO2 groups compared with F1B hamsters, but to a significantly greater extent in those fed the high PUFA diet compared with the standard or high saturated fat diet.

Conclusion These results show that a high intake of saturated fat improves survival in heart failure compared with a high PUFA diet or low-fat diet, despite persistent mitochondrial defects.

Keywords Cardiomyopathy • Low-carbohydrate diet • Metabolism • Obesity

1. Introduction

Little is known about the impact of dietary lipid on the development and progression of heart failure.[1] Treatment guidelines for heart failure patients provide no specific recommendations regarding fat intake and suggest that heart failure patients follow the dietary guidelines provided for the prevention of coronary heart disease (CHD).[2–4] Recent studies suggest that high-fat/low-carbohydrate diets in the absence of obesity exert favourable effects on cardiac function and survival in rodent models of heart failure induced by infarction,[5–8] chronic hypertension,[9–14] or genetic cardiomyopathy.[15] Clinical studies have yet to address this issue; however, there is an extensive literature on dietary fat and CHD, with recent evidence showing no increase with greater intake of fat (including saturated fat) and reduced risk of CHD with high intake of polyunsaturated fatty acids (PUFA).[16] This has prompted a paradigm shift for the prevention of coronary disease, with emphasis now on increased PUFA intake and decreased consumption of refined carbohydrate.[17] At present, the dietary recommendation for heart failure patients in terms of fat intake is not clear.

Recent evidence suggests that there might be major differences between the intake of saturated fatty acids and PUFA in the

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development and progression of heart failure, although there has not been a direct comparison. In rodents with heart failure induced by aortic constriction or myocardial infarction, a high-fat diet rich in saturated fatty acids either improved or had no adverse effect on cardiac function and survival compared with a standard low-fat diet.[5–12,18] On the other hand, a high intake of the n-3 PUFA α-linolenic acid (18:3n3) combined with a low intake of linoleic acid (18:2n6) prolonged survival in hamsters with dilated heart failure caused by δ-sarcoglycan deficiency.[15] Feeding spontaneously hypertensive heart failure rats a high-fat diet comprised mainly of long-chain saturated fat shortens survival; however, treatment with a similar amount of fat that was mainly linoleic acid prolonged survival compared with a standard low-fat diet.[14] This effect was associated with improved mitochondrial function and greater linoleic acid incorporation into mitochondrial membranes. Linoleic acid has been associated with pro-inflammatory effects via elongation and desaturation to form arachidonic acid, the precursor to prostanoids, while ω-3 PUFA such as α-linolenic acid inhibit this process. Dietary PUFA and saturated fatty acids also have divergent effects on gene expression, apoptosis, cell signalling, and mitochondrial function and could differentially affect the progression of heart failure.[19] Feeding rats a high-fat diet comprised of saturated fatty acids (16:0 and 18:0) may be pro-apoptotic compared with linoleic acid.[19] Thus, a diet rich in linoleic acid might improve or maintain mitochondrial function, prevent cardiomyocyte apoptosis, and improve cardiac function and survival in heart failure.

The goal of this study was to assess the effects of high-fat diets rich in PUFA or saturated fat on survival, mitochondrial function, and phospholipid fatty acid composition in a genetic model of heart failure. We hypothesized that a high PUFA diet (45% of energy from fat) enriched with both α-linolenic and linoleic acid would improve mitochondrial oxidative capacity, prevent Ca^{2+}-induced mitochondrial permeability transition pore (MPTP) opening, and prolong survival compared with either a standard low-fat diet or a high-fat diet enriched with palmitate and stearate. Previous studies addressing the role of dietary fat in the development and progression of heart failure used rodent models with relatively short-term arterial hypertension or myocardial infarction in young animals.[5–7,9–13,18] Here, we wished to assess the long-term effects of high-fat intake (6–15 months) on survival and biochemical mechanisms and we wanted to avoid the high variability and expense encountered with surgical models or high sodium intake. Thus, we used the δ-sarcoglycan null cardiomyopathic hamster, a well-established rodent model of dilated cardiomyopathy that has decreased mitochondrial oxidative capacity and has been shown to respond to nutritional and metabolic therapies.[15,20,21]

2. Methods

2.1 Experimental design

The animal protocol was approved by the University of Maryland School of Medicine Institutional Animal Care and Use Committee and conducted according to the Guidelines for the Care and Use of Laboratory Animals (National Institutes of Health publication 85-23). Investigators were blinded to treatment when measurements were performed. The animals were maintained on a 12 h light/dark cycle, and all procedures were performed between 2 and 5 h from the start of the light phase. Male δ-sarcoglycan null cardiomyopathic hamsters (Bio TO2 strain) were acquired at 5 weeks of age from Bio Breeder Inc. (Watertown, MA, USA) and 1 week later were assigned to either a standard high-carbohydrate/low-fat diet, a high PUFA/low-carbohydrate diet, or a high saturated fat/low-carbohydrate diet (*Table 1*). Healthy age-matched male F1B hamsters were used as a control group and were fed only the standard diet. Animals were housed six per cage and given food and water *ad libitum*.

Table 1 Macronutrient content and fatty acid composition of the diets (expressed as a per cent of total energy in the chow)

	Standard (%)	High PUFA (%)	High saturated fat (%)
Protein	20	20	20
Carbohydrate	68	35	35
Fat	12	45	45
Palmitate(16:0)	2.8	3.6	10.9
Palmitoleate (16:1n7)	0.2	0.1	1.2
Stearate (18:0)	2.5	1.2	9.1
Oleate (18:1n9)	4.4	13.5	17.8
Linoleate (18:2n6)	1.8	20.7	3.1
α-Linolenoate (18:3n3)	0.2	6.0	0.3

Two series of experiments were performed: the 'survival protocol' assessed the effects of diet on mortality, and the 'physiological evaluation protocol' determined the impact of diet on cardiac and mitochondrial function prior to fully advanced cardiomyopathy and accelerated mortality (30 weeks of age). The survival protocol compared the standard low-fat diet with the high PUFA and high saturated fat diets. Since previous studies with Bio TO2 strain found a median survival of 37–46 weeks of age, with 100% death by 55–75 weeks,[22–24] we set the maximum length of treatment at 78 weeks, after which any surviving animals were euthanized. Six-week-old TO2 hamsters were assigned to either the standard diet ($n = 35$), high PUFA diet ($n = 30$), or high saturated fat diet ($n = 30$). A control group of F1B hamsters were fed a standard diet ($n = 17$). There animals were weighed weekly and otherwise were not handled.

The physiological evaluation protocol assessed the effects of 24 weeks of dietary treatment (from 6 to 30 weeks of age). Animals were assigned to either the standard, high PUFA, or high saturated fat diet ($n = 11$ or 12/group) and after 23 weeks underwent an echocardiogram. The following week, they underwent a terminal study to assess cardiac biochemistry, histology, and mitochondria function.

2.2 Diets

The diets were custom-manufactured using purified ingredients (Research Diets, New Brunswick, NJ, USA). All diets contained 20% of energy from protein (casein + L-cystine) (*Table 1*). The carbohydrate sources were maltodextrin (12% of total energy) and corn starch (55% in standard diet and 22% in high-fat diets). The fat source for the standard diet was a mixture of lard, cocoa butter, and soybean oil totalling 12% of total energy. The total fat content was 45% of total energy in both the PUFA and saturated fat diets. The fat sources in the high PUFA diet were safflower, flaxseed, olive, and soybean oils, which generated a diet high in both linoleic acid (18:2n6) and α-linolenic acid, as both of these PUFA are frequently consumed at high levels in humans[25,26] and are effective at prolonging survival in rodent models of heart failure.[14,15] The high saturated fat diet consisted of lard, cocoa butter, and soybean oil.

2.3 Echocardiography

In the physiological evaluation protocol, cardiac function was assessed after 23 weeks of treatment using echocardiography (Vevo 770, Visual Sonics, Toronto, Canada) with a 15 MHz linear array transducer (model 716). Hamsters were anaesthetized with 2% isoflurane in oxygen and placed on a warming pad. Adequacy of anaesthesia was monitored by lack of reflex response to foot pinch. Two-dimensional cine loops and guided M-mode frames were recorded from the parasternal short axis. Absolute wall thickness was calculated as the sum of diastolic posterior and anterior wall thicknesses, and relative wall thickness as the ratio of absolute wall thickness to end-diastolic diameter. Fractional shorting was calculated as (end-diastolic diameter − end-systolic diameter)/end-diastolic diameter.

2.4 Terminal surgery

After 24 weeks of treatment, the hamsters in the physiological evaluation protocol were anaesthetized with 5.0% isoflurane between 3 and 6 h after initiation of the light phase while given free access to food. Adequacy of anaesthesia was monitored by lack of reflex response to foot pinch. The thorax was opened and blood was collected from the left ventricle (LV) and immediately placed on ice tubes containing EDTA, and centrifuged at 4°C to obtain plasma. The heart was removed, and sections of the LV free wall were taken for biochemical analysis and histology and stored at −80°C, and the remainder was used for mitochondrial isolation. Epididymal and retroperitoneal fat and lungs were removed.

2.5 Mitochondrial isolation

Mitochondria were isolated as described previously.[27] Briefly LV tissue (~250 mg) was minced and homogenized in 1:10 cold modified Chappel–Perry buffer (100 mM KCl, 50 mM MOPS, 5 mM $MgSO_4$, 1 mM EGTA, 1 mM ATP, and 0.2 mg/mL BSA), and centrifuged at 500 *g*. Subsequent centrifugation separated and purified subsarcolemmal mitochondria (SSM). Separation of the interfibrillar mitochondria (IFM) was achieved by incubation on ice with trypsin (5 mg/g wet mass) for 10 min and subsequent centrifugations.

2.6 Mitochondrial respiration

Mitochondrial oxygen consumption was assessed in SSM and IFM.[27,28] Isolated mitochondria (0.5 mg mitochondrial protein/mL) were respired in buffer containing 100 mM KCl, 50 mM MOPS, 5 mM KH_2PO_4, 1 mM EGTA, and 1 mg/mL BSA. State 3 and 4 respiration was measured with glutamate + malate (10 and 5 mM, respectively) and succinate + rotenone (10 mM and 7.5 μM, respectively). State 4 respiration was measured with oligomycin (4 μM). The mitochondrial diameter of IFM and SSM was measured by flow cytometry, as described previously.[29]

2.7 Mitochondrial Ca^{2+} retention

The ability of mitochondria to retain Ca^{2+}, an index of MPTP opening, was assessed in isolated mitochondria as described previously in detail.[27] Briefly, using a 96-well fluorescence plate reader, 25 μg of mitochondrial protein was suspended in 200 μL of respiration buffer without BSA but with 5 μM EGTA and 1 mM $MgCl_2$. Glutamate and malate were added as substrates (10 and 5 mM, respectively), and extramitochondrial Ca^{2+} was monitored using 1 μM calcium green (5N) and fluorescence measured at 485 and 538 nm for excitation and emission wavelengths, respectively, at 37°C. Ca^{2+} was added in increments of 25 nmol/mg mitochondrial protein at 7 min intervals.

2.8 Metabolic and biochemical parameters

Plasma glucose and free fatty acids were measured by spectrophotometric enzymatic assays and insulin by ELISA.[11] Maximal activities of medium-chain acyl-CoA dehydrogenase and citrate synthase were measured spectophotometrically.[11]

2.9 Histology

Histological analysis was performed on LV tissue from the lateral free wall for myocyte cross-sectional area, interstitial fibrosis, and cardiomyocyte apoptosis as described previously.[11]

2.10 Fatty acid analysis

Myocardial phospholipid fatty acid composition was assessed in homogenates of the right ventricle by gas chromatography coupled with mass spectrometry according to a modification of the transesterification method as described previously.[30] The same method was used to analyse the diets. The double bond index (DBI) was calculated as the sum of the concentration of each fatty acid times the number of double bonds.

2.11 Statistical analysis

Data are presented as mean ± the standard error. Differences among groups in the measurements from the physiological evaluation protocol were determined using a non-parametric one-way ANOVA with a Dunn's *post hoc* test for multiple comparisons. Data for extramitochondrial Ca^{2+} from the retention assay and body mass were evaluated using a two-way ANOVA with a Holm–Sidak *post hoc* test appropriate. Survival was assessed with a Kaplan–Meier analysis. A value of $P < 0.05$ was considered significant.

3. Results

3.1 Survival

The median survival on treatment increased from 278 days with the standard diet to 361 days with high saturated fat ($P < 0.01$) (*Figure 1*). The high PUFA group was not different from the standard diet (260 days), but was shorter than the high saturated fat group ($P < 0.01$).

3.2 Body and organ mass

After 24 weeks of treatment in the physiological evaluation protocol, there were no significant differences among the three diets in the mass of the whole body or LV (*Table 2*) or in wet or dry lung mass or liver mass (data not shown). Fat pad mass was higher with high PUFA diet compared with the standard or high saturated fat diets. The lung wet/dry mass ratio was not different between the healthy F1B hamsters compared with the TO2 hamsters (data not shown), consistent with well-compensated cardiac function at this time point. More prolonged treatment resulted in significant and equivalent

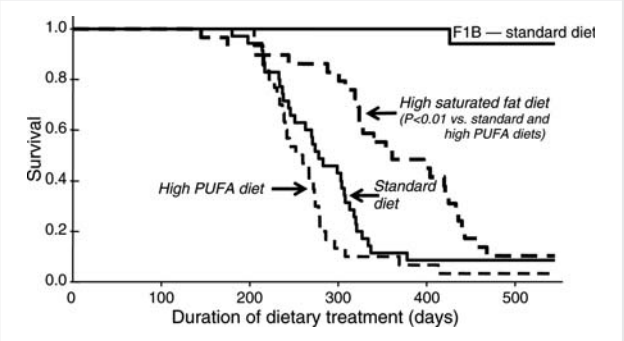

Figure 1 Animal survival plotted as a function of duration of dietary treatment. Hamsters initiated dietary treatment at 6 weeks of age.

Table 2 Body and organ mass, and echocardiographic and histological data after 24 weeks of dietary treatment after 24 weeks of treatment from the physiological evaluation protocol

	Normal F1B	Cardiomyopathic TO2 hamsters		
	Standard diet	Standard diet	High PUFA	High SAT
Body mass (g)—6 weeks old	77.8 ± 1.5	69.4 ± 2.1*	68.8 ± 3.7*	72.7 ± 1.8
Body mass (g)—30 weeks old	166.4 ± 4.5	131.3 ± 3.3§	136.9 ± 4.5§	134.2 ± 4.9§
Retroperitoneal fat pad mass (g)	1.23 ± 0.06	0.52 ± 0.03§,†	0.89 ± 0.07§	0.52 ± 0.06§,†
Epididymal fat pad mass (g)	3.1 ± 0.17	1.4 ± 0.07*	2.2 ± 0.15‡	1.6 ± 0.2*
LV mass (mg)	395 ± 9	304 ± 8§	317 ± 12§	321 ± 7§
LV mass/body mass (mg/g)	2.38 ± 0.05	2.32 ± 0.04	2.32 ± 0.05	2.41 ± 0.09
LV mass/tibia length (mg/mm)	15.1 ± 0.3	12.3 ± 0.2§	13.1 ± 0.4§	12.8 ± 0.3§
LV end-diastolic diameter (cm)	0.47 ± 0.02	0.50 ± 0.02	0.45 ± 0.05	0.50 ± 0.09
LV end-systolic diameter (cm)	0.29 ± 0.03	0.38 ± 0.02	0.32 ± 0.02	0.37 ± 0.03
LV fractional shortening	0.39 ± 0.02	0.26 ± 0.03*	0.29 ± 0.02*	0.28 ± 0.02*
Absolute wall thickness (cm)	0.37 ± 0.01	0.29 ± 0.01*	0.31 ± 0.01*	0.28 ± 0.01*
Relative wall thickness	0.80 ± 0.04	0.59 ± 0.03*	0.69 ± 0.04	0.57 ± 0.03*
Myocyte cross-sectional area (μm^2)	603 ± 25	665 ± 26	622 ± 27	635 ± 18
Interstitial fibrosis (% area)	9.8 ± 1.0	10.6 ± 0.5	9.7 ± 0.75	10.4 ± 0.7
Cardiomyocyte apoptosis (DNAfr/10 000 myocytes)	2.3 ± 0.8	5.2 ± 1.3	3.1 ± 0.6	3.6 ± 0.5

*$P < 0.05$ vs. F1B.
§$P < 0.001$ vs. F1B.
†$P < 0.001$ vs. TO2 high PUFA.
‡$P < 0.05$ vs. TO2 standard diet.

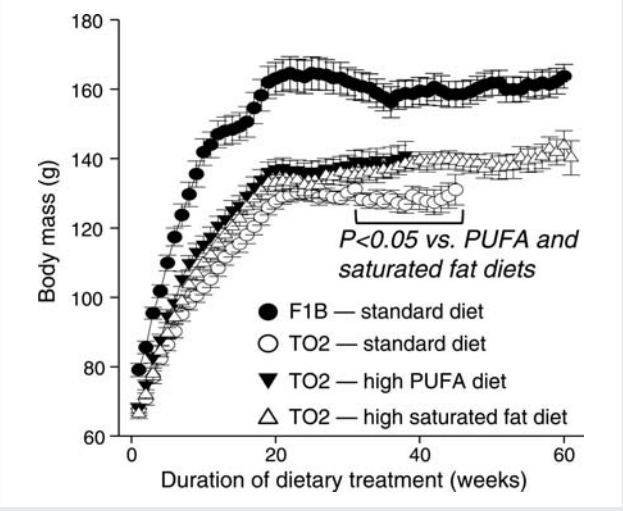

Figure 2 Body mass from the survival protocol assessed weekly from initiation of the diet until 67% mortality was achieved in a treatment group.

increases in body mass of ~10% after ~30 weeks in the high PUFA and saturated fat groups (*Figure 2*).

3.3 Cardiac function and histology

Dietary fat intake did not affect LV systolic or diastolic diameters, fractional shortening, or absolute or relative wall thickness (*Table 2*). Cardiomyocyte apoptosis was low in both the F1B and TO2 hamsters on the standard diet and was unaffected by the high-fat diets (*Table 2*). Interstitial fibrosis and cardiomyocyte cross-sectional area were similar among groups (*Table 2*).

3.4 Metabolic measures

Plasma glucose was higher in the cardiomyopathic hamster on the standard and high PUFA diets compared with the normal F1B hamsters on the standard diet, but there were no differences in glucose concentration among the three diet groups in cardiomyopathic hamsters (*Table 3*). Plasma insulin concentrations were not affected by the high PUFA or saturated fat diets (*Table 3*). The plasma free fatty acid concentration was elevated by the high PUFA diet, with no other difference among groups.

3.5 Mitochondrial enzymes and function

The activity of the mitochondrial marker enzymes citrate synthase (citric acid cycle) and medium-chain acyl-CoA dehydrogenase (fatty acid β-oxidation) was significantly reduced to a similar extent in all three TO2 groups compared with the normal F1B animals. Consistent with this was a significant decrease in mitochondrial yield in the TO2 hamsters compared with the F1B that was primarily in the IFM subpopulation (*Table 4*). There were no differences in mitochondrial respiratory parameters, or the size of SSM or IFM as assessed by flow cytometry, among any of the groups (*Table 4*). The cardiomyopathic animals had a lower medium-chain acyl-CoA dehydrogenase/citrate synthase activity ratio, consistent with a greater down-regulation of the capacity for fatty oxidation capacity relative to citric acid cycle flux (*Figure 3*). Activation of peroxisome proliferator-activated receptor α (PPARα) by a high-fat diet or a pharmacological agonist increases the oxidative capacity for fat relative to the citric acid

Table 3 Metabolic data after 24 weeks of dietary treatment

	Normal F1B	Cardiomyopathic TO2 hamsters		
	Standard diet	Standard diet	High PUFA	High SAT
Plasma glucose (mg/dL)	110 ± 4	137 ± 6*	166 ± 17*	135 ± 9
Plasma insulin (ng/mL)	1.2 ± 0.3	1.0 ± 0.2	1.0 ± 0.2	0.7 ± 0.3
Plasma free fatty acids (mM)	0.50 ± 0.07	0.67 ± 0.07	1.23 ± 0.2*	0.73 ± 0.09
Urine thromboxane B_2 (pg/μmol creatine)	22.8 ± 5.9	25.6 ± 6.6	33.2 ± 6.4	25.1 ± 3.2

Samples were taken in unfasted animals given free access to food and water.
*$P < 0.05$ vs. F1B.

Table 4 Function of isolated cardiac mitochondria after 24 weeks of dietary treatment

	Normal F1B	Cardiomyopathic TO2 hamsters		
	Standard diet	Standard diet	High PUFA	High SAT
Subsarcolemmal mitochondrial				
Yield (mg mito prot./g wet mass)	6.0 ± 0.6	4.1 ± 0.3*	5.9 ± 0.5‡	5.2 ± 0.4
Mitochondria diameter (nm)	619 ± 22	558 ± 11	555 ± 20	565 ± 20
Glutamate + malate: State III	148 ± 10	148 ± 17	143 ± 9	124 ± 18
Glutamate + malate: State IV	42.2 ± 2.6	41 ± 4.5	38 ± 2.7	38 ± 4.8
Glutamate + malate: RCR	7.4 ± 1.5	10.3 ± 2.8	8.6 ± 2.2	10.6 ± 4.1
Succinate + rotenone: State III	296 ± 16	258 ± 15	260 ± 24	244 ± 9.5
Succinate + rotenone: State IV	108 ± 8.4	119 ± 13	93 ± 6	102 ± 6.6
Succinate + rotenone: RCR	4.1 ± 0.4	5.1 ± 0.5	4.4 ± 0.7	3.9 ± 0.5
Interfibrillar mitochondria				
Yield (mg mito prot./g wet mass)	9.0 ± 0.6	4.1 ± 0.6*	4.6 ± 0.5*	4.0 ± 0.5*
Mitochondria diameter (nm)	572 ± 19	560 ± 14	555 ± 15	584 ± 17
Glutamate + malate: State III	201 ± 26	154 ± 23	190 ± 28	175.1 ± 16
Glutamate + malate: State IV	60 ± 6	53 ± 8.4	63 ± 9	60 ± 8
Glutamate + malate: RCR	10 ± 4.4	5 ± 0.7	5 ± 1	6 ± 0.8
Succinate + rotenone: State III	357 ± 28	281 ± 20	329 ± 22	339 ± 28
Succinate + rotenone: State IV	122 ± 11	132 ± 11	137 ± 12	150 ± 11
Succinate + rotenone: RCR	3.8 ± 0.4	4.0 ± 0.3	3.6 ± 0.3	4.8 ± 0.9

*$P < 0.05$ vs. F1B.
‡$P < 0.05$ vs. TO2 standard diet.

cycle or electron transport chain.[5,31] The lack of effect of the high-fat diets on the ratio of medium-chain acyl-CoA dehydrogenase to citrate synthase activity suggests that the high PUFA and high saturated fat diets did not activate PPARα sufficiently to elevate the capacity for fatty acid oxidation.

Heart failure induced by severe myocardial injury alters mitochondria in a manner that causes greater susceptible to stress-induced MPTP opening.[32,33] Here, we observed a decrease in the capacity of isolated mitochondria to take up exogenous Ca^{2+}, an index of enhanced MPTP opening. As shown in *Figure 4*, IFM, but not SSM, showed a higher extramitochondrial [Ca^{2+}] for a given cumulative Ca^{2+} load in all three TO2 groups compared with the F1B group. The PUFA diet further increased the sensitivity of IFM to Ca^{2+}-induced MPTP opening compared with the TO2 hamsters fed the standard or high saturated fat diets. There were no effects of diet or strain in SSM (*Figure 4*).

3.6 Cardiac phospholipid fatty acid composition

Compared with the F1B strain, the cardiomyopathic TO2 animals had a significant increase in stearic acid, eicosapentaenoic acid, docosahexaenoic acid, and the sum of n-3 PUFA, and a decrease in oleic acid (*Figure 5* and *Table 5*).

Among the TO2 hamsters, there were only modest differences between the standard diet and the high saturated fat group, as the only significant differences were a small decrease in eicosapentaenoic acid and the sum of n-6 PUFA in the high saturated fat group (*Figure 5* and *Table 5*). On the other hand, the high PUFA diet had a profound effect on cardiac phospholipid fatty acid composition, as seen in a ~60% increase in linoleic acid and a doubling in α-linolenic acid (*Figure 5* and *Table 5*), both of which were highly enriched in the diet (*Table 1*). The increase in PUFA in cardiac phospholipids resulted

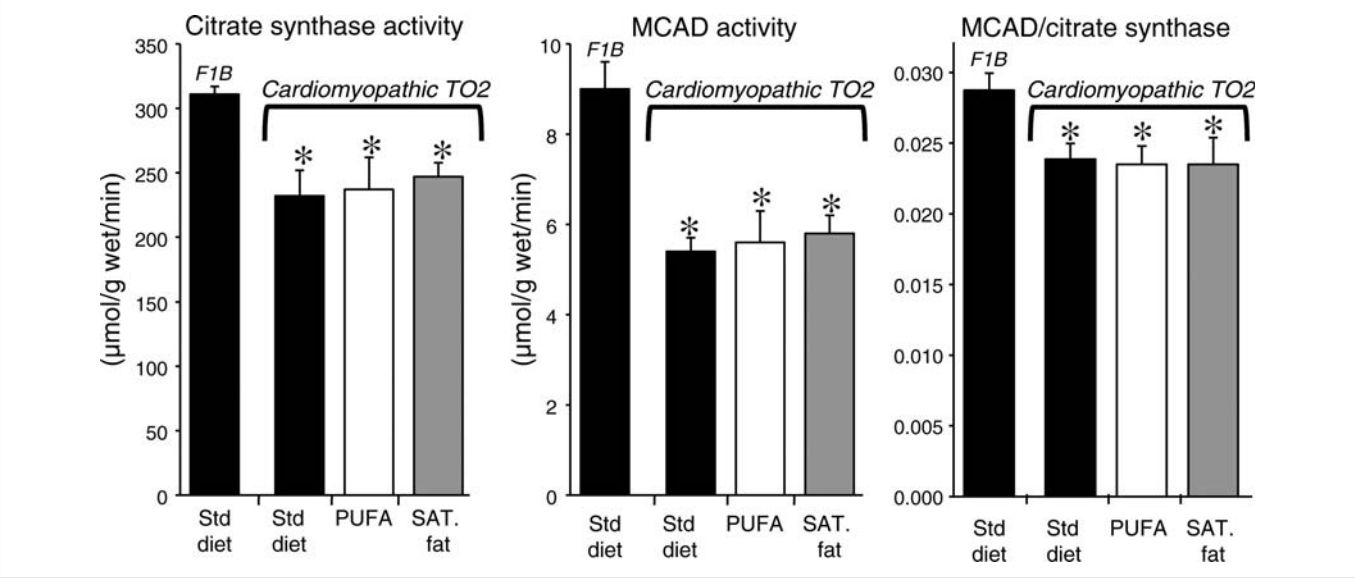

Figure 3 Myocardial activity of the mitochondrial oxidative enzymes citrate synthase and medium-chain acyl-CoA dehydrogenase, and the ratio of medium-chain acyl-CoA dehydrogenase to citrate synthase. *$P < 0.05$ vs. F1B.

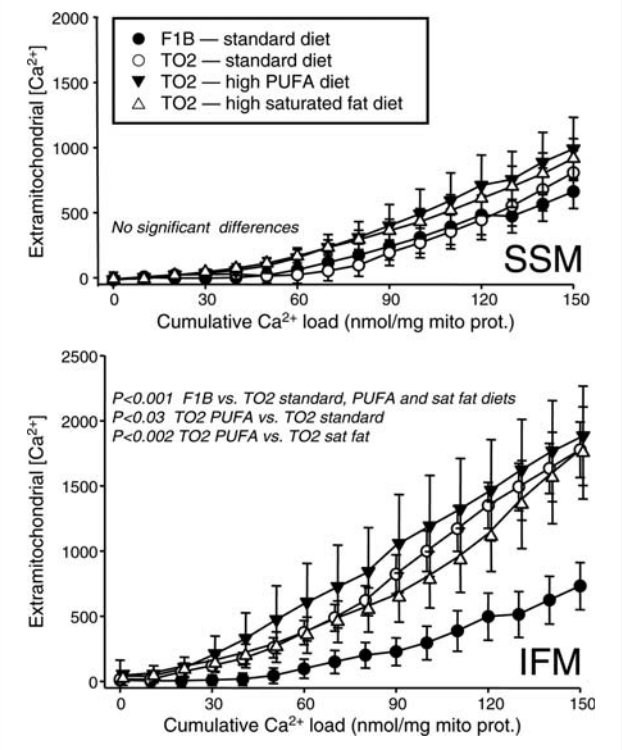

Figure 4 Extramitochondrial [Ca^{2+}] plotted as a function of the cumulative Ca^{2+} added to SSM (top panel) and IFM (lower panel).

in a significant increase in the DBI compared with all other groups (*Table 5*).

The high PUFA diet resulted in a significant reduction in oleic acid and the sum of monounsaturated fatty acids (oleic acid + palmitoleic acid) compared with the standard diet or high saturated fat group ($P < 0.05$). This occurred despite relatively similar oleic acid content in the two high-fat diets (*Table 1*). On the other hand, there were no differences between the two high-fat diets in docosahexaenoic acid, the main n-3 PUFA in cardiac phospholipids, or the sum of n-3 PUFA.

Treatment with the high PUFA diet decreased arachidonic acid compared with the TO2 animals on the standard and high saturated fat diets (*Figure 5* and *Table 5*). Since the PUFA diet was high in both linoleic acid and α-linolenic acid (18:3n3), the fall in arachidonic acid was presumably due to inhibition of the elongation of linoleic acid by elevated n-3 PUFA. There were no differences in the thromboxane B2/creatinine ratio in urine, an index of systemic inflammatory prostaglandin production, which suggests there were no major alterations in prostanoid production.

4. Discussion

While we hypothesized that a high PUFA diet enriched with both α-linolenic and linoleic acid would improve mitochondrial oxidative capacity, prevent Ca^{2+}-induced MPTP opening, and prolong survival compared with a standard low-fat diet or a high saturated fat diet, our results were very much to the contrary. Surprisingly, consumption of the diet high in long-chain saturated fatty acids prolonged life compared with a standard low-fat diet. This clear beneficial effect was not due to correction of myocardial mitochondrial oxidative capacity or greater susceptibility to MPTP opening, nor to improved cardiac pump function. In contrast to our hypothesis, a high PUFA fat diet enriched with α-linolenic acid and linoleic acid from vegetable sources did not improve survival, cardiac mechanical function, or mitochondrial energetics or resistance to MPTP opening. In contrast to previous studies in rodents that found a beneficial effect on survival when diets high in α-linolenic acid and linoleic acid were given separately to rodents with heart failure,[14,15] our findings suggest that high intake of both α-linolenic acid and linoleic acid has a negative effect.

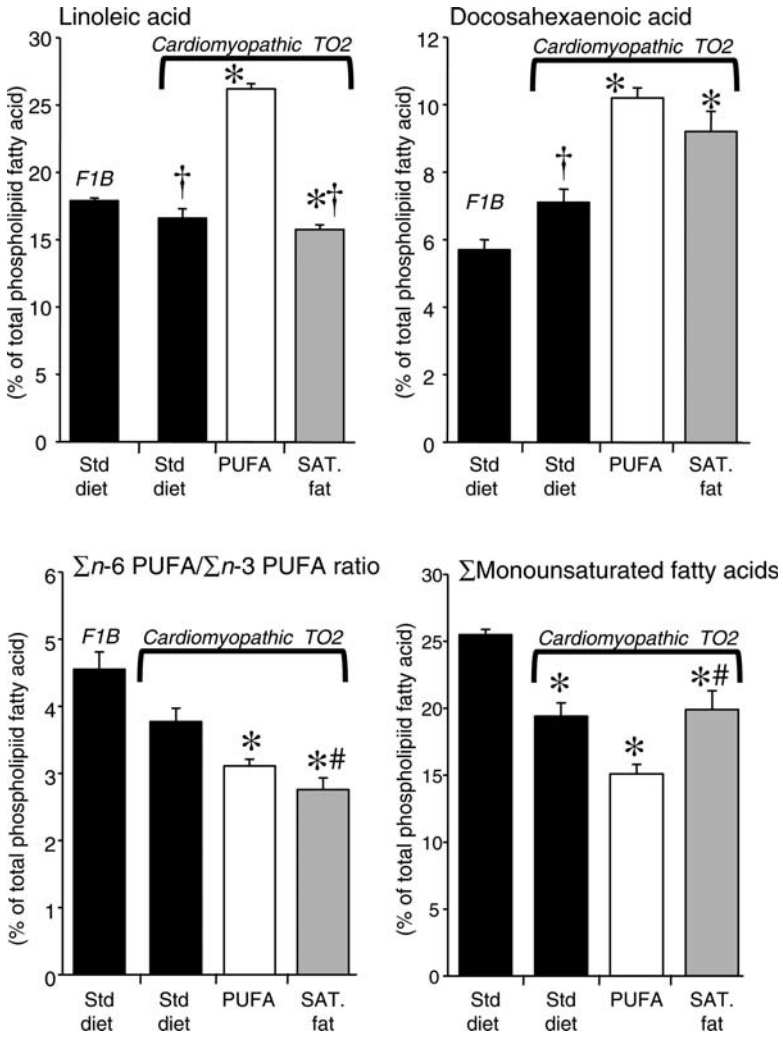

Figure 5 Myocardial phospholipid fatty acid composition expressed as a per cent of total fatty acids (top), and the ratio of the sum of n-6 PUFA/n-3 PUFA (bottom left), and the sum of monounsaturated fatty acids (right) from the right ventricular myocardium after 24 weeks of dietary treatment. $^{*}P < 0.05$ vs. F1B; $^{\S}P < 0.001$ vs. F1B; $^{\dagger}P < 0.05$ vs. TO2 on the high PUFA diet; $^{\#}P < 0.05$ vs. TO2 of the standard diet.

The lack of improvement in survival with the high PUFA diet compared with the high saturated fat diet was associated with a 75% increase in plasma free fatty acids with the PUFA diet compared with the standard and high saturated fat groups. Clinical studies in the 1960s found an association between elevated free fatty acids and ventricular arrhythmias,[34] and it was later shown that plasma fatty acid concentration is a strong predictor of sudden cardiac death.[35] Experimental evidence showed that elevated free fatty acids can prevent normal sarcolemmal ion handling and could potentially trigger fatal arrhythmias.[36] Thus, it is possible that elevated free fatty acids triggered arrhythmias and sudden death in the PUFA group.

The two high-fat diets were low in carbohydrate compared with the standard diet (*Table 1*), as protein content was constant across diets. We were careful to use a standard diet free of sugars, as we previously observed that high sugar intake can accelerated the development of heart failure in pressure overload models.[1] This does not seem to be the case in cardiomyopathic hamsters, as we recently observed in a parallel study that high sugar intake did not adversely affect cardiac function or survival compared with the standard diet (P.A.H., W.C.S. *et al.*, unpublished observation). Thus, the beneficial effect of the high saturated fat diet does not appear to be due to the reduction in 'glycaemic load' that accompanied reduced carbohydrate intake.

Examination of the fatty acid profile of cardiac phospholipids revealed only subtle differences between the high saturated fat diet and the standard diet groups, but a dramatic difference in survival. These findings suggest that the difference in survival between these two groups is not due to changes in membrane fatty acid composition. On the other hand, when one compares the high PUFA and high saturated fat groups, there is a striking elevation in linoleic acid and depletion of oleic acid and total monounsaturated fatty acids (*Figure 5* and *Table 5*), suggesting that these alterations may have contributed to the failure of the high PUFA diet to improve survival. A similar relationship between the cardiac oleic acid content and life span was observed in normal mice, although mice have a very low level of monounsaturated fatty acid (~5–7%) in the myocardium compared with hamsters (~20%) or humans (~14%).[37] It is important to note that our analysis of total tissue phospholipid fatty acid

Table 5 Myocardial phospholipid fatty acid composition after 24 weeks of dietary treatment

	Normal F1B	Cardiomyopathic TO2 hamsters		
	Standard diet	Standard diet	High PUFA	High SAT
Palmitic acid (16:0)	18.7 ± 0.2	18.9 ± 0.4†	14.2 ± 0.3*‡	16.6 ± 0.4*
Palmitoleic acid (16:1n7)	1.03 ± 0.06	0.91 ± 0.03	0.46 ± 0.09*	0.54 ± 0.04
Stearic acid (18:0)	20.3 ± 0.4	23.9 ± 0.7*	23.8 ± 0.4*	26.2 ± 1.0*
Oleic acid (18:1n9)	22.4 ± 0.4	16.6 ± 0.9*	13.6 ± 0.7*	18.3 ± 1.3†
Vaccenic acid (18:1n7)	2.01 ± 0.12†	1.88 ± 0.06†	1.06 ± 0.02	1.12 ± 0.01
Linoleic acid (18:2n6)	17.9 ± 0.2	16.6 ± 0.7†	26.2 ± 0.4*	15.8 ± 0.3*,†
α-Linolenic acid (18:3n3)	0.12 ± .01†	0.16 ± .0.1†	0.31 ± 0.01	0.14 ± 0.01†
Arachidonic acid (20:4n6)	10.0 ± 0.2	12.2 ± 1.0†	8.3 ± 0.2*	10.3 ± 0.5†
Eicosapentaenoic acid (20:5n3)	0.29 ± 0.01	0.42 ± 0.02*	0.41 ± 0.02*	0.35 ± 0.02‡
Docosapentaenoic acid (22:5n3)	0.40 ± 0.01	0.50 ± 0.02	0.57 ± 0.02	0.53 ± 0.03
Docosahexaenoic acid (22:6n3)	5.7 ± 0.3	7.1 ± 0.4†	10.2 ± 0.3*	9.2 ± 0.6*
∑Saturated fatty acids	39.2 ± 0.3	42.3 ± 0.6*	38.1 ± 0.4‡	42.9 ± 1.1*,#
∑Monounsaturated fatty acids	25.4 ± 0.4	19.4 ± 1.0*	15.1 ± 0.7*	19.9 ± 1.4*,#
∑n-3 PUFA	6.5 ± 0.3	8.2 ± 0.4	11.4 ± 0.4‡,*	10.2 ± 0.6*
∑n-6 PUFA	28.9 ± 0.2	30.0 ± 0.6	35.4 ± 0.3*	27.0 ± 0.4‡,#
Double bond index	142.0 ± 1.7	153.0 ± 4.0	170.0 ± 2.0*‡	155.2 ± 4.5*

*$P < 0.05$ vs. F1B.
‡$P < 0.05$ vs. TO2 standard diet.
†$P < 0.001$ vs. TO2 high PUFA.
#$P < 0.05$ vs. TO2 high PUFA.

composition provides a gross profile of fatty acyl moieties in pooled myocardial membrane. Future studies should assess the effects of dietary lipid in heart failure on isolated myocardial membrane fractions (i.e. sarcolemmal, sarcoplasmic reticulum, mitochondria) and the fatty acyl composition of distinct lipid classes (phosphotidylethanolamine, phosphotidylcholine, cardiolipin, etc.).

The elevation in linoleic acid in myocardial phospholipids with the PUFA diet compared with both the standard and high saturated fat diets could accelerate inflammation if it increased arachidonic acid (20:4n6), the precursor to inflammatory prostaglandins. Arachidonic acid is highly abundant in cardiac phospholipids, and it has been suggested that diets high in linoleic acid will accelerate conversion to arachidonic acid and production of inflammatory prostanoids. This was not observed, presumably because conversion of linoleic acid to arachidonic acid was inhibited by high intake of α-linolenic acid. Together, these finding suggest that the improved survival in the high saturated fat group compared with the PUFA diet was not mediated via changes in inflammatory prostaglandins.

IFM from the cardiomyopathic hamster showed a clear increase in sensitivity to Ca^{2+}-induced MPTP opening compared with normal animals; however, this defect was not observed in SSM (*Figure 4*). Thirty years ago, Hoppel *et al.*[21] showed a defect in oxidative phosphorylation in IFM but not SSM in cardiomyopathic hamsters. Here, we extend this finding, showing that greater sensitivity to Ca^{2+}-induced MPTP opening is also isolated to IFM. While diet had no effect in SSM, in IFM, the high PUFA diet further increased susceptibility to permeability transition. While we observed a trend for a lower state 3 respiration rate IFM in cardiomyopathic animals, it was not statistically significant, but our mitochondrial protein yield was decreased by ∼50%. This is in contrast to Hoppel *et al.*, who observed similar mitochondrial yields in normal and cardiomyopathic hamsters, but decreased state 3 respiration. This difference could perhaps be due to strain, as they used the Bio 14.6 strain while we used the Bio TO2, though we both used the same vendor. In any case, available evidence suggests a clear defect in mitochondrial function in this model that is specific to IFM.

Limitations to the present investigation need to be addressed. First, we did not assess the effects of the high PUFA or high saturated fat diets in normal hamsters. There is a clear possibility that what is good for the failing heart might be bad for the normal healthy myocardium. We previously assessed the effects of various high PUFA and high saturated fat diets in normal rats and mice and observed no major differences in cardiac function or structure,[9–12,18] though we did observe elevated myocardial palmitoylceramide content and cardiomyocyte apoptosis in normal rats fed a high saturated fat diet compared with a high n-6 PUFA diet.[19] A similar increase in apoptosis was not observed in cardiomyopathic hamsters in the present study, suggesting that the differences might be due to species or to the underlying effects of heart failure. A second limitation is that we measured the fatty acid profile in total cardiac phospholipids, which does not reflect the complexity and biological activities of the various pools of individual phospholipid classes in specific organelles. Future studies need to isolate subcellular membrane fractions and identify the composition of the various phospholipid subclasses. Another limitation is the lack of assessment of cardiac function and myocardial pathology at a later time point (e.g. 40–50 weeks) when mitochondrial pathology and cardiomyocyte apoptosis are further advanced.[21] Lastly, we did not assess the electrocardiogram or cause of death in the present study. The improved survival with high saturated fat compared with high PUFA may be due to greater

ventricular arrhythmias and cardiac sudden death. In addition, the precise cause of death with the standard diet is unclear in the model. Thus, future studies should employ telemetry to evaluate possible diet-induced changes in cardiac electrophysiology and to determine whether death is due to ventricular arrhythmias.

In summary, our results show that consuming a diet high in saturated fat prolongs life compared with a standard low-fat diet in an established genetic model of heart failure. This appeared to be independent of mitochondrial function. In contrast, consuming a high PUFA fat diet rich in α-linolenic acid and linoleic acid did not improve survival nor LV or mitochondrial function. The failure of the high PUFA diet to improve survival in this model was associated with an increase in linoleic acid and a decrease in oleic acid in cardiac phospholipids, suggesting that future studies should pursue a thorough analysis of the phospholipid composition in various cardiac membrane compartments in an effort to unmask the underlying causes for the differences in survival.

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