Diets Rich in Saturated and Polyunsaturated Fatty Acids: Metabolic Shifting and Cardiac Health

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OBJECTIVE: The aim of this study was to determine the effects of diets rich in saturated and polyunsaturated fatty acids on metabolic pathways and the relation of metabolic shifting to oxidative stress in cardiac tissue.

METHODS: Male Wistar rats (age, 60 d; $n = 10$) were fed with a control low-fat diet, a diet rich in saturated fatty acids (SFAs), or a diet rich in polyunsaturated fatty acids (PUFAs). After 5 wk of treatment, sera were used for protein and lipid determinations. Protein, glycogen, triacylglycerol, lactate dehydrogenase, citrate synthase, β-hydroxyacyl coenzyme-A dehydrogenase, catalase, glutathione peroxidase, superoxide dismutase, lipoperoxide, and lipid hydroperoxide were measured in cardiac tissue.

RESULTS: The SFA group had higher triacylglycerol, cholesterol, low-density lipoprotein cholesterol, and atherogenic index (ratio of cholesterol to high-density lipoprotein) than did the PUFA and control groups. The PUFA group had low serum cholesterol, triacylglycerol, and low-density lipoprotein cholesterol as compared with the SFA group. SFA increased myocardial lipid hydroperoxide and diminished glutathione peroxidase. Despite the beneficial effects on serum lipids, the PUFA diet led to the highest levels of myocardial lipoperoxide and lipid hydroperoxide and diminished superoxide dismutase and catalase activities. The PUFA effects were related to increased feed efficiency, increased susceptibility to lipoperoxidation, and metabolic shifting in cardiac tissue. PUFA elevated triacylglycerol levels and decreased myocardial glycogen concentrations. The ratios of lactate dehydrogenase to citrate synthase and β-hydroxyacyl coenzyme-A dehydrogenase to citrate synthase were increased, indicating myocardial reduction of tricarboxylic acid cycle.

CONCLUSIONS: PUFAs have been recommended as a therapeutic measure in preventive medicine to lower serum cholesterol, but PUFAs increased oxidative stress in the heart by providing cardiac susceptibility to lipoperoxidation and shifting the metabolic pathway for energy production. The control diet, which was much lower in calories and fat, produced better overall clinical outcomes, better fat profiles, and less oxidative stress than did the diets rich in fatty acids. *Nutrition* 2004;20:230–234. ©Elsevier Inc. 2004

KEY WORDS: polyunsaturated fatty acid, saturated fatty acid, diets, heart, oxidative stress, lipids, serum

INTRODUCTION

In many societies, fats are considered part of the basic food supply and may have long-term, low-intensity, negative consequences. The Western diet generally includes at least 30% to 40% of energy as fat, and a typical American diet is 20% fat, resulting in a 40% energy intake from lipid sources having saturated fatty acids (SFAs) and polyunsaturated fatty acids (PUFAs).[1] Current recommendations are to increase dietary vegetable oils as a therapeutic interaction measure with a significant role in preventive medicine to increase the ratio of PUFAs to SFAs, lower serum cholesterol and, thus indirectly, prevent atherosclerosis.[2] However, it is questionable whether the increase in PUFA consumption, despite the decrease in serum cholesterol concentration, might really be safe.

The types of fat in the diet determine the relative composition of biomembranes, and PUFAs are indeed substrates for free radical reactions leading to lipoperoxidation.[3] Lipoperoxidation is an important event of reactive oxygen species (ROS) and oxidative stress causing cell death.[4] Indeed, the use of oxygen in the oxidative metabolism of fuel results in ROS production,[5] and alterations in food constituents or fuel for energy generation may be related to oxidative stress.[6,7] Therefore, oxidative stress not only may be associated with unsaturated fatty acid content in biomembranes but also may be dependent on metabolic pathway shifting for energy production.

Although recent investigations have established that ROS may be an important contributor to cardiac dysfunction and myocardial damage,[8,9] there is little information on the metabolic effects of dietary fatty acids and on cardiac evaluating markers of oxidative stress. The effectiveness of dietary PUFAs to improving beneficial effect on oxidative stress in cardiac tissue is not presently known.

Thus, the major purpose of the present study was to investigate the metabolic effects of diets rich in PUFA and SFA in cardiac tissue. The effects of diets rich in SFA and PUFA on myocardial oxidative stress may be dependent on metabolic pathways and fuel utilization for energy production.

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TABLE I.

FATTY ACIDS COMPOSITION OF DIETARY FATS

Fatty acid*	C	SFA	PUFA
C6		0.86	
C8		11.79	
C10		10.79	
C12	1.55	17.01	
C14	1.99	5.88	0.30
C16	43.51	33.63	35.08
C16:1 (ω-9)	0.54	0.21	0.27
C18	2.32	1.77	1.71
C18:1 (ω-9)	10.92	10.13	14.71
C18:2 (ω-6)	36.83	8.28	46.99
C18:3 (ω-3)	2.34	0.26	0.95
Total saturated	49.37	81.73	37.09
Total monounsaturated	11.46	10.34	14.98
Total polyunsaturated	39.17	8.54	47.94
Total unsaturated	50.63	18.88	62.92
Metabolizable energy (kcal/g)	12.5	22.0	22.0

* Grams per 100 g of fat.
C, control diet; PUFA, diet rich in polyunsaturated fatty acid; SFA, diet rich in saturated fatty acid.

MATERIALS AND METHODS

Animals

The Ethical Committee for Conduction of Animal Studies at the Institute of Biological Sciences, University of São Paulo State, approved the experimental protocol, and all animals were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care as outlined in the *Guide to the Care and Use of Experimental Animals*. Thirty 60-d-old male Wistar rats weighing 180 to 200 g were individually housed in polypropylene cages in an environmentally controlled, clean-air room with a temperature of 22°C ± 3, 12-h light-and-dark cycle, and a relative humidity of 60% ± 5. The rats received water ad libitum and were randomly assigned to one of three groups (n = 10/group). Rats in the control group (C) were given free access to a low-fat diet consisting of Purina rodent chow containing 26.5% protein, 3.8% fat, 40.0% carbohydrate, 4.5% crude fiber, and 3.0 kcal/g of metabolizable energy (3074 SIF, Purina Ltd., Campinas, São Paulo, Brazil). Rats in the SFA group received a diet enriched with 25% SFAs (26.5% protein, 28.8% fat, 40.0% carbohydrate, 4.5% dietary fiber, and 5.25 kcal/g of metabolizable energy). Rats in the PUFA group received a diet enriched with 25% PUFAs (26.5% protein, 28.8% fat, 40.0% carbohydrate, 4.5% dietary fiber, and 5.25 kcal/g of metabolizable energy).

Preparation of Diets

Diets were prepared by mixing the supplemented ingredients with a previously triturated Purina chow. Therefore, all diets provided sufficient amounts of vitamins, minerals, essential amino acids, and lipids. Casein was added to the diets enriched with fatty acids to achieve the same protein content as the C chow. The fat-enriched diets were formulated by mixing proportions of coconut oil with corn oil. To produce an SFA-enriched diet, 1000 mL of corn oil was mixed with 1240 mL of coconut oil; to produce a PUFA-enriched diet, 1000 mL of coconut oil was mixed with 1240 mL of corn oil.[10] The proportion of the different types of fatty acids was analyzed with gas chromatography (17A-Simatzu, Tokyo, Japan; Table I). Diets were given fresh each day, and pellets and food consumption were measured daily at the same time (9:00 to 10:00 AM). Body weights were determined once a week.

Experimental Procedure

After 5 wk of treatment, rats were fasted overnight (12 to 14 h) and killed by decapitation. Blood was placed into a centrifuge tube and allowed to clot to obtain the serum. The serum was analyzed for total protein,[11] albumin,[12] cholesterol,[12] high-density lipoprotein (HDL) cholesterol,[13] low-density lipoprotein (LDL) cholesterol,[14] and triacylglycerol.[12]

The heart was removed, and the cardiac adipose tissue was discarded. The heart was weighed, and the left ventricle (LV) was separated out and divided into three parts. In this experiment, the metabolic determination was done on LVs (myocytes) free of external adipose tissue. LV samples of 200 mg were homogenized in 0.6 M of perchloric acid, and the amount of free glucose was determined with a glucose oxidase procedure (Test Kit CELM, Modern Laboratory Equipment Company, São Paulo, Brazil). Tissue glycogen was then hydrolyzed to glucose directly in homogenates by treatment with amyloglucosidase (Sigma, St. Louis, MO, USA), and the total glucose released was measured. Glycogen concentration was calculated as the difference between total and free glucose.[15] The triacylglycerols were extracted with chloroform:methanol at a ratio of 2:1[16] from the second piece of the LV.

LV samples of 200 mg were weighed and homogenized in 5 mL of a cold phosphate buffer (0.1 M, pH7.4) containing 1 mM of ethylenediaminetetraacetic acid (EDTA). Tissue homogenates were prepared in a motor-driven Teflon-glass Potter-Elvehjem, tissue homogenizer (1 min, 100 rpm) immersed in ice water. The homogenate was centrifuged at 10 000 rpm for 15 min. The supernatant was analyzed for total protein, lipoperoxide, lipid hydroperoxide, reduced glutathione (GSH), lactate dehydrogenase (LDH; E.C.1.1.1.27.), β-hydroxyacyl coenzyme-A dehydrogenase (OHADH; E.C.1.1.1.35.), citrate synthase (CS; E.C.4.1.3.7.), glutathione peroxidase (GSH-Px; E.C.1.11.1.9.), superoxide dismutase (SOD; E.C.1.15.1.1.), and catalase (E.C.1.11.1.6.). Catalase was determined with phosphate buffer (pH 7.0) at 240 nm.[17]

The assay medium for LDH contained 50 mM of Tris-HCl buffer (pH 7.5), 0.15 mM of nicotinamide adenine dinucleotide in reduced form, and 1 mM of pyruvate (omitted for the C group).[18] For CS activity the assay medium consisted of 50 mM of Tris-HCl (pH 8.1), 0.3 mM of acetyl-coenzyme A, 0.1 mM 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB), and 0.5 mM oxaloacetate (omitted for the C group).[18] OHADH was assayed in a medium containing 50 mM of Tris-HCl (pH7.0), 5 mM of EDTA, 0.45 mM of reduced nicotinamide adenine dinucleotide, and 0.1 mM of acetoacetyl-coenzyme A.[18]

Lipid hydroperoxide was determined with 100 μL of sample and 900 μL of a reaction mixture containing 0.25 mM of $FeSO_4$, 25 mM of H_2SO_4, 0.1 mM of xylenol orange, and 4 mM of butylated hydroxytoluene in 90% (v/v) methanol. The mixtures were incubated for 30 min at room temperature before measurement at 560 nm.[19] Lipoperoxide was measured colorimetrically by thiobarbituric acid.[20] Malondialdehyde standard was prepared from 20 mM of 1,1,3,3-tetraethoxypropane. After incubation at 37°C for 60 min, the samples were poured in test tubes containing 1 mL of 20% trichloroacetic acid. One milliliter of 0.67% thiobarbituric acid, in 2 M of sodium sulfate, was added. After 15 min of boiling and subsequent cooling, the samples were centrifuged for 10 min at 3000 rpm and the optical density of the supernatant fraction was read at 530 nm.

GSH was determined in 0.4 M Tris Buffer, pH 8.9, with DTNB after previous treatment with 50% trichloroacetic acid. The absorbance was read within 5 min of the addition of DTNB at 412 nm. Standard curves were obtained with 1 mM of GSH.[21]

GSH-Px was assayed by using 0.15 M of phosphate buffer (pH 7.0) containing 5 mM of EDTA, 0.1 mL of 0.0084 M nicotinamide

adenine dinucleotide phophate, 0.005 mL of oxidized glutathione reductase, 0.01 mL of 1.125 M NaN_3 (sodium aside), and 0.1 mL of 0.15 M GSH.[22] SOD activity was determined based on the ability of the enzyme to inhibit the reduction of nitroblue tetrazolium, which was generated by 37.5 mM of hydroxylamine (Carlo Erba, Milan, Italy) in alkaline solution.[23] The assay was performed in 0.5 M of sodium carbonate with EDTA. The reduction of nitroblue tetrazolium by (O_2^-) to blue formazan was measured at 560 nm by using an enzyme-linked immunosorbent assay reader (Bio-Tech Instruments, Inc., Winooski, VT, USA). The rate of nitroblue tetrazolium reduction in the absence of tissue was used as the reference rate. One unit of SOD was defined as the amount of protein needed to decrease the reference rate to 50% of maximum inhibition. All data were expressed in units of SOD/mg of protein.

The spectrophotometric determinations were performed in an Pharmacia Biotech spectrophotometer (model 974213; Cambridge, UK). The absorptivity of reduced nicotinamide adenine dinucleotide at 340 nm was 6.22 μM/cm and that of DTNB at 412 nm was 13.6 mM/cm. All reagents were from Sigma.

Based on food intake and the amount of calories,[24] the following parameters were calculated:

$$\text{voluntary food intake (\%)} = (\text{mean food consumption} \times 100)/(\text{mean body weight})$$

$$\text{feed efficiency (g/g)} = (\text{mean body weight gain})/(\text{mean food consumption})$$

Statistical Analysis

The results are expressed as means ± standard error of the mean. Significance of difference was tested by analysis of variance and Tukey's test to compare treatment groups with each other, not just with the control. $P = 0.05$ was considered statistically significant.[25]

RESULTS

Fatty Acid Composition of the Diets, Body Weight, Energy Intake, and Feed Efficiency

The SFA had 81.73% SFAs and 18.88% PUFAs. The PUFA diet had 62.92% PUFAs and 37.09% SFAs (Table I). The PUFA diet contained the highest proportion of ω-6 fatty acids, and the SFA diet contained more short-chain SFAs. The SFA diet contained the lowest proportion of the ω-6 fatty acids. No alterations were observed in the amount of metabolizable energy of the PUFA and SFA diets.

Table II shows that dietary fat supplementation increased final body weight, body weight gain, and energy intake but decreased voluntary food intake. SFA rats had high food and energy intakes compared with PUFA rats. However, final body weight and body weight gain were significantly higher in PUFA than in SFA animals. PUFA rats had higher feed efficiency than SFA rats.

Serum Determinations

No alterations were observed in serum total protein concentrations, but albumin was highest in PUFA animals. SFA rats had high triacylglycerol, cholesterol, LDL cholesterol, and atherogenic index (ratio of cholesterol to HDL cholesterol) than did PUFA and C rats. PUFA rats had lower triacylglycerol, cholesterol, and LDL cholesterol concentrations than did SFA rats. HDL cholesterol was higher and the ratio of cholesterol to HDL was lower in PUFA than in SFA rats (Table III).

TABLE II.

EFFECTS OF DIETS ON FINAL BODY WEIGHT, BODY WEIGHT GAIN, MEAN FOOD CONSUMPTION, VFI, ENERGY INTAKE, AND FE*

Parameters	Diets		
	C	SFA	PUFA
Initial body weight (g)	200.1 ± 2.7	200.3 ± 1.8	201.0 ± 2.3
Final body weight (g)	317.5 ± 3.2‡§	331.4 ± 4.1†§	352.7 ± 5.2†‡
Body weight gain (g)	116.4 ± 1.2‡§	131.4 ± 2.7†§	153.7 ± 1.5†‡
Mean food consumption (g/d)	38.2 ± 0.2‡§	36.1 ± 0.3†§	34.7 ± 0.2†‡
VFI (%)	12.1 ± 0.2‡§	10.9 ± 0.8†	9.84 ± 1.6†
Energy intake (kcal/d)	114.6 ± 0.6‡§	189.5 ± 1.6†§	182.2 ± 1.0†‡
FE (g/g)	8.71 ± 0.31‡§	10.39 ± 0.07†§	12.65 ± 0.40†‡

* Values are mean ± standard error of the mean.
† Significantly different from C group, $P < 0.05$.
‡ Significantly different from SFA group, $P < 0.05$.
§ Significantly different from PUFA group, $P < 0.05$.
C, control diet; FE, feed efficiency; PUFA, diet rich in polyunsaturated fatty acid; SFA, diet rich in saturated fatty acid; VFI, voluntary food intake

Myocardial Analysis

Table IV summarizes the biochemical determinations in cardiac tissue. PUFA induced decreased SOD and catalase activities, whereas lipoperoxide and hydroperoxide were increased in cardiac tissue. GSH-Px activity was decreased in SFA rats, whereas hydroperoxide was increased in these animals. PUFA decreased CS cardiac activity. The ratios of OHADH to CS and LDH to CS were significantly increased in the PUFA group. No alterations were observed in LDH, OHADH, and CS activities and in the ratios of LDH to CS and OHADH to CS compared with C and SFA rats. No alterations were observed in total protein and GSH concentrations

TABLE III.

EFFECTS OF DIETS ON TOTAL PROTEIN, ALBUMIN, TRIACYLGLYCEROL, CHOLESTEROL, HDL CHOLESTEROL, LDL CHOLESTEROL, AND CHOLESTEROL:HDL RATIO IN SERUM*

Biochemical determinations	Diets		
	C	SFA	PUFA
Total protein (g/dL)	4.8 ± 0.3	5.2 ± 0.4	5.5 ± 0.2
Albumin (g/dL)	2.8 ± 0.4§	3.2 ± 0.3§	4.0 ± 0.4†‡
Triacylglycerol (mg/dL)	68.0 ± 7.3‡	82.5 ± 18.0†§	61.7 ± 7.9‡
Cholesterol (mg/dL)	80.3 ± 9.5‡	150.4 ± 17.9†§	111.4 ± 11.9‡
HDL cholesterol (mg/dL)	33.8 ± 2.2‡	30.3 ± 3.2†§	35.0 ± 2.4‡
LDL cholesterol (mg/dL)	30.1 ± 4.3‡§	97.6 ± 10.4†§	68.1 ± 4.3†‡
Cholesterol:HDL ratio	2.37 ± 0.12‡	4.89 ± 0.18†§	3.01 ± 0.39‡

* Values are mean ± standard error of the mean.
† Significantly different from C group, $P < 0.05$.
‡ Significantly different from SFA group, $P < 0.05$.
§ Significantly different from PUFA group, $P < 0.05$.
C, control diet; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PUFA, diet rich in polyunsaturated fatty acid; SFA, diet rich in saturated fatty acid.

TABLE IV.

EFFECTS OF DIETS ON CARDIAC ANALYSIS*

Biochemical determinations	Diets		
	C	SFA	PUFA
Lipoperoxide (nM/g tissue)	79.3 ± 10.8§	101.1 ± 15.2§	200.3 ± 13.4†‡
Hydroperoxide (nM/g tissue)	162.5 ± 11.3‡§	178.4 ± 4.99†§	190.7 ± 4.2†‡
GSH (μM/g tissue)	1.3 ± 0.1	1.5 ± 0.2	1.7 ± 0.2
SOD (U/mg protein)	26.8 ± 2.6§	28.6 ± 2.3§	12.1 ± 1.8†‡
GSH-Px (U/mg tissue)	21.1 ± 2.3‡	15.4 ± 1.2†§	22.3 ± 2.3‡
Catalase (U/g tissue)	8.2 ± 2.1§	8.2 ± 2.4§	3.5 ± 0.3†‡
LDH (U/g tissue)	320.6 ± 12.9	326.0 ± 32.2	320.0 ± 17.8
CS (U/100 mg tissue)	7.0 ± 0.1§	8.0 ± 0.2§	4.0 ± 0.1†‡
OHADH (U/100 mg tissue)	3.0 ± 0.2	3.0 ± 0.1	3.1 ± 0.1
OHADH:CS	0.42 ± 0.03§	0.38 ± 0.15§	0.75 ± 0.84†‡
LDH:CS	45.8 ± 1.3§	40.7 ± 3.2§	80.0 ± 1.8†‡
Total protein (g%)	24.0 ± 2.5	23.1 ± 1.5	24.5 ± 3.1
Glycogen (g%)	2.6 ± 0.4§	2.4 ± 0.3§	1.4 ± 0.1†‡
Triacylglycerol (mg%)	0.2 ± 0.002§	0.3 ± 0.01§	0.5 ± 0.01†‡

* Values are mean ± standard error of the mean.
† Significantly different from C group, $P < 0.05$.
‡ Significantly different from SFA group, $P < 0.05$.
§ Significantly different from PUFA group, $P < 0.05$.
C, control group; CS, citrate synthase; GSH, reduced glutathione; GSH-Px, glutathione peroxidase; LDH, lactate dehydrogenase; OHADH, β-hydroxyacyl coenzyme-A dehydrogenase; PUFA, diet rich in polyunsaturated fatty acid; SFA, diet rich in saturated fatty acid; SOD, superoxide dismutase

in cardiac tissue. The cardiac glycogen concentrations were significantly low in PUFA animals. PUFA rats had the highest myocardial triacylglycerol concentrations.

DISCUSSION

Dietary fat has been implicated as a major factor promoting excess energy intakes and obesity.[1] However, it has been suggested that not all fats may have the same effect on obesity.[26] Table II shows that high-fat diets reduced voluntary food intake, even though final body weight and body weight gain were increased in these animals. This is in agreement with previous reports.[6,9] Several factors might contribute to differential patterns of voluntary food intake. The hypophagia is due in part to the energy density of the high-fat diet (average, 5.25 kcal/g) as compared with the C diet (3.0 kcal/g). It is interesting to note that, when rats were presented with high-fat diets, they ate significantly less than the C rats (Table II). The percentages of energy derived from fat, carbohydrate, and protein were, respectively, 49.4%, 30.5%, and 20.1% for the high-fat diets and 11.3%, 53.4%, and 35.3% for the C diet. There is much evidence that important stimuli for the control of food intake and energy balance are produced by the circulating energy pool that consists mainly of lipids.[27] In contrast, although SFA rats had higher food and energy intakes than did PUFA rats, they showed lower body weight gain. Therefore, final body weight and body weight gain were related not only to the amount of food consumed but also to the quality of the fat in each diet.

SFA and PUFA diets had the same caloric content (Table I), but PUFA rats had increased feed efficiency, body weight gain, and final body weight than did SFA rats (Table II). This study clearly demonstrated that the type and structure of lipids ingested can have very different metabolic consequences. Hence, the effects of fat diets may be dependent on which type of fatty acid is used in dietary supplementation. Isoenergetic substitution of medium-chain triacylglycerols for long-chain triacylglycerols in a high-fat diet, reduced energy intake and medium-chain fatty acids are more rapidly oxidized, especially by the liver, and are less rapidly deposited in adipose tissue than are long-chain fatty acids.[26] The SFA diet had a higher concentration of medium-chain fatty acids than did the PUFA diet (Table I), and SFA rats had a lower body weight gain than did PUFA rats. The PUFA rats had the highest albumin concentration, and albumin is a plasmatic free-fatty acid carrier (Table III).

In the present study, feeding rats with the PUFA-enriched diet diminished serum triacylglycerol, cholesterol, and LDL cholesterol levels as compared with the SFA diet. HDL cholesterol was higher and the ratio of cholesterol to HDL was lower in PUFA than in SFA rats. These findings are in accordance with other studies, which indicated that a PUFA-rich diet has beneficial effects on risk factors for atherosclerosis.[2,28] Although rats have a relative small amount of LDL,[29] Table III shows that, in C rats under fasting conditions, more HDL cholesterol was found, but that SFA and PUFA rats had a significant increase in the LDL fraction. It is obvious that the C diet, which was much lower in calories and fat (Table I), had overall better clinical outcomes and better fat profiles. Table III also shows that LDL cholesterol and the ratio of cholesterol to HDL were lowest in the C animals.

There are other consequences related to high-fat dietary intake, such as increased ROS and alteration on markers of oxidative stress in cardiac tissue. Lipoperoxide was increased in PUFA rats, and lipid hydroperoxide concentrations were increased in SFA and PUFA rats (Table IV). Lipoperoxide and lipid hydroperoxide are widely studied as markers of lipoperoxidation.[4] The use of oxygen and the oxidative metabolism of fuels result in free radical production. Oxygen-derived radicals are cytotoxic to cells. During oxidative stress, ROS, superoxide anion (O_2^-), hydroxyl radicals ($OH^\bullet$), and hydrogen peroxide (H_2O_2) can elicit widespread damage to cell constituents such as membrane lipids. There is evidence that mitochondria are major production sites and a primary target for ROS during the course of oxidative phosphorylation. Some investigators believe that, under physiologic conditions, fatty acids act as mild uncouplers preventing pronounced changes in transmembrane potential that could lead to ROS formation.[5,26] The uncoupling effect of fatty acids is greater for PUFAs and considerably mild for short-chain SFAs.[26,30] Depolarization of inner mitochondrial membrane fatty acids is important because of its consequences for energy metabolism and cell death. Depolarization causes impaired adenosine triphosphate synthesis and greater ROS, which can then promote lipoperoxidation.[4]

As expected, the C rats fed with less fat and fewer calories had decreased oxidative stress (Table IV). The observation that SFA rats had lower myocardial hydroperoxide concentrations than did PUFA animals demonstrates the importance of the PUFA:SFA ratio on lipoperoxidation. The readiness with which fatty acids peroxidize is proportional to the number of double bonds, and a positive correlation between the amount of PUFA in the diet and the rate of microsomal lipoperoxidation has been demonstrated in rats.[3] These observations support the concept that the sensitivity of the cardiac tissue to oxidative stress may depend on dietary factors and, therefore, that the oxidative stability of the cardiac cells is determined by the balance between factors such as PUFAs, which change the PUFA:SFA ratio in the membrane and enhance the sensitivity to lipoperoxidation and the levels of antioxidants.

SOD catalyzes the destruction of O_2^- by dismutation and hydrogen peroxide formation. Catalase and GSH-Px catalyze the conversion of hydrogen peroxide to water. Although no alterations had been observed in GSH-Px, PUFA rats had diminished SOD and catalase activities. No alterations were observed in SOD and

catalase activities, whereas GSH-Px was decreased in SFA rats (Table IV).

The most obvious effects of fatty acids on cardiac function were their actions on metabolic pathways. The decreased CS activities in PUFA rats clearly indicated that PUFAs induced more lipoperoxidation and cardiac cell membrane alterations than did SFAs. CS is the key enzyme for the control of the flux of metabolites through the tricarboxylic acid cycle, OHADH for fatty acid oxidation, and LDH for lactate production by anaerobic glycolysis.[18] Although no alterations were observed in LDH and OHADH, the LDH:CS ratio was increased in PUFA rats, indicating increased glycolytic pathway relative to aerobic metabolism. The OHADH:CS ratio was highest in PUFA rats, indicating decreased tricarboxylic acid cycle in relation to fatty acid degradation. Although no alterations were observed in total protein, PUFAs had a dramatic effect on the heart, as evidenced by triacylglycerol accumulation in cardiac tissue (Table IV).

The glycogen concentration was decreased in PUFA rats (Table IV). Glycogen concentration appears to be an important factor for reducing cardiac damage.[31] Cardiac glycogen is a potential source of myocardial energy and is reserved for hypoxic emergencies.[32]

The significance of this study lies in the potential application of its findings to human nutrition and preventive medicine. Unfortunately, difficulties arise in attempting to determine whether oxidative stress arises from a high level of energy intake or is dependent on metabolic component of the diet, or both. Hence, studies designed to investigate the causes of oxidative stress from metabolic pathway and fuel utilization for energy production are difficult and may be impossible to undertake in human subjects. Therefore, much of the basis research in this area has been performed in laboratory animals. Direct evidence for food intake, fat oxidation, and oxidative stress induction in cardiac tissue is available only in experimental animals. However, it is difficult to compare studies using dietary fatty acids. Even if the same dietary source was employed in different studies, the content of the different fats may differ extensively.

In this study, we clearly demonstrated that changes in dietary fatty acids affect cardiac oxidative stress. Because the majority of the metabolic conditions leading to cardiac dysfunction remain clinically silent and become manifest only when cardiac alteration is effectively installed, a high-fat diet can have long-term effects on cardiac heath. Nevertheless, despite its effects on serum lipids, diets rich in PUFAs may be disadvantageous because it can lead to the highest cardiac oxidative stress. This observation may be of pathophysiologic relevance, especially in view of the importance of oxidative stress to the alteration processes in the heart that result in further adverse effects.

In conclusion, diets rich in SFAs and PUFAs increased oxidative stress in cardiac tissue. The C diet, which was much lower in calories and fat, had better overall clinical outcome, better fat profile, and produced less oxidative stress than did diets rich in fatty acids. By taking advantage of the oxidative stress markers, we were able to show that diets rich in PUFAs, despite the beneficial effects on serum lipids, were deleterious as compared with SFAs in the heart by providing cardiac susceptibility to lipoperoxidation and shifting the metabolic pathway for energy production.

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