Differential effects of saturated and unsaturated fatty acid diets on cardiomyocyte apoptosis, adipose distribution, and serum leptin

Isidore C. Okere, Margaret P. Chandler, Tracy A. McElfresh, Julie H. Rennison, Victor Sharov, Hani N. Sabbah, Kou-Yi Tserng, Brian D. Hoit, Paul Ernsberger, Martin E. Young, and William C. Stanley. Differential effects of saturated and unsaturated fatty acid diets on cardiomyocyte apoptosis, adipose distribution, and serum leptin. *Am J Physiol Heart Circ Physiol* 291: H38–H44, 2006. First published January 27, 2006; doi:10.1152/ajpheart.01295.2005.—Fatty acids are the primary fuel for the heart and are ligands for peroxisome proliferator-activated receptors (PPARs), which regulate the expression of genes encoding proteins involved in fatty acid metabolism. Saturated fatty acids, particularly palmitate, can be converted to the proapoptotic lipid intermediate ceramide. This study assessed cardiac function, expression of PPAR-regulated genes, and cardiomyocyte apoptosis in rats after 8 wk on either a low-fat diet (normal chow control (NC); 10% fat calories) or high-fat diets composed mainly of either saturated (Sat) or unsaturated fatty acids (Unsat) (60% fat calories) (n = 10/group). The Sat group had lower plasma insulin and leptin concentrations compared with the NC or Unsat groups. Cardiac function and mass and body mass were not different. Cardiac triglyceride content was increased in the Sat and Unsat groups compared with NC (P < 0.05); however, ceramide content was higher in the Sat group compared with the Unsat group (2.9 ± 0.2 vs. 1.4 ± 0.2 nmol/g; P < 0.05), whereas the NC group was intermediate (2.3 ± 0.3 nmol/g). The number of apoptotic myocytes, assessed by terminal deoxynucleotide transferase-mediated dUTP nick-end labeling staining, was higher in the Sat group compared with the Unsat group (0.28 ± 0.05 vs. 0.17 ± 0.04 apoptotic cells/1,000 nuclei; P < 0.04) and was positively correlated to ceramide content (P < 0.02). Both high-fat diets increased the myocardial mRNA expression of the PPAR-regulated genes encoding uncoupling protein-3 and pyruvate dehydrogenase kinase-4, but only the Sat diet upregulated medium-chain acyl-CoA dehydrogenase. In conclusion, dietary fatty acid composition affects cardiac ceramide accumulation, cardiomyocyte apoptosis, and expression of PPAR-regulated genes independent of cardiac mass or function.

Fatty acids are the primary metabolic fuel for the heart and also act as endogenous ligands to the peroxisome proliferator-activated receptors (PPARs) and induce genes that encode proteins that regulate fatty acid metabolism (35). Studies (8) in transgenic mice show that overexpression of PPARα reduces the capacity for fatty acid oxidation and leads to cardiomyocyte-restricted PPARα deletion in mice sensitive rats fed a high-fat diet compared with normal low-fat chow despite similar levels of hypertension. Furthermore, cardiomyocyte-restricted PPARα or PPARβ/δ deletion in mice reduces the capacity for fatty acid oxidation and leads to cardiomyocyte lipid accumulation, LV hypertrophy, and contractile dysfunction (3, 4, 31). These findings suggest that exaggeration or diminution of fatty acid metabolism causes a low saturated fat/high-carbohydrate diet for optimal cardiac health (18). On the other hand, results of recent studies suggest that consumption of a low-carbohydrate/high-fat diet can be effective for weight loss (22, 30). At present, there is little information regarding the effects of prolonged high-fat diets on the heart.

Lipid accumulation in the heart has been observed under conditions of elevated plasma free fatty acids, such as Type 2 diabetes mellitus and chronic high-fat feeding (10, 11, 16, 17, 32), and is linked with cardiomyocyte apoptosis, ventricular (LV) hypertrophy, and contractile dysfunction (5). Excessive storage of metabolically active lipid products in the heart can lead to the formation of ceramide, a proapoptotic lipid intermediate formed primarily from palmitate (4, 31). Intracellular lipid accumulation and consequent apoptosis are dependent on the type of fatty acid in the milieu: studies in isolated cells show that palmitate induces apoptosis but oleate does not and that the addition of oleate to medium containing palmitate protects against apoptosis (14, 21).

Fatty acids are the primary metabolic fuel for the heart and also act as endogenous ligands to the peroxisome proliferator-activated receptors (PPARs) and induce genes that encode proteins that regulate fatty acid metabolism (35). Studies (8) in transgenic mice show that overexpression of PPARα is associated with myocardial accumulation of triglyceride and ceramide, accelerated fatty acid oxidation, LV hypertrophy and dilatation, and eventual systolic contractile dysfunction. A similar effect is observed with cardiac-specific overexpression of fatty acyl-CoA synthase (5), suggesting that accelerated fatty acid uptake by the heart can result in “cardiac lipidotoxicity” (4). On the other hand, our laboratory (26) recently observed improved LV contractile function and attenuation of LV hypertrophy and remodeling in hypertensive Dahl salt-sensitive rats fed a high-fat diet compared with normal low-fat chow despite similar levels of hypertension. Furthermore, cardiomyocyte-restricted PPARα or PPARβ/δ deletion in mice reduces the capacity for fatty acid oxidation and leads to cardiomyocyte lipid accumulation, LV hypertrophy, and contractile dysfunction (3, 4, 31). These findings suggest that exaggeration or diminution of fatty acid metabolism causes...
myocardial hypertrophy and dysfunction. On the other hand, our laboratory (24) recently found that feeding a high-fat diet or administration of a PPARα agonist does not affect LV function or remodeling in rats with infarct-induced heart failure. This neutral impact occurred despite upregulation of the fatty acid metabolic pathway and accumulation of myocardial triglyceride.

Little is known about the effects of high-fat diets on cardiac function, apoptosis, and the expression of PPAR-regulated genes in normal animals. In addition, the effects of potential differences in long-chain saturated fatty acids and unsaturated fatty acids have not been distinguished. In the present study, we assessed the effects of feeding rats either standard low-fat chow (10% of calories from fat as 3% unsaturated and 7% saturated fatty acids) or high-fat diets (60% of calories from fat) composed primarily of either saturated or unsaturated fatty acids. We hypothesized that a diet high in saturated fatty acids would increase myocardial ceramide content, trigger apoptosis, and result in cardiac dysfunction, whereas a high-fat diet composed of unsaturated fatty acids would lack this effect. We further hypothesized that the expression of PPAR-regulated genes would be increased by a saturated or unsaturated high-fat diet. Studies were performed in normal Wistar rats subjected to 8 wk of dietary treatment. Rats were fed ad libitum; however, there were no differences among groups in body weight, and thus the confounding effects of obesity observed in previous feeding studies were avoided (23, 39, 40). LV function was assessed by echocardiography and direct measurements of LV pressure. Because insulin and the adipokine hormones leptin and adiponectin can affect cardiac metabolism and arterial blood pressure and potentially trigger cardiac hypertrophy (7, 33, 41), we also assessed the effects of the high-fat diets on regional fat deposits and circulating hormone levels.

**METHODS**

Experimental design. All procedures for this study were conducted according to Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996) and were approved by the Institutional Animal Care and Use Committee of the Case Western Reserve University. All measurements were performed with the investigator blinded to treatment. Twenty-eight male Wistar rats were obtained at 8–9 wk of age from Harlan (Indianapolis, IN) and maintained on a reverse 12:12-h light-dark cycle. The high-fat diets used in the study were obtained from Research Diets (New Brunswick, NJ).

Initial pretreatment measurements were made for systolic arterial blood pressure, body weight, and LV function. Systolic pressure was measured by using the tail-cuff method after habituation to the test procedure. The rats were randomized into three groups: a normal chow control group (NC; n = 9), a high long-chain saturated fatty acid diet group (Sat; n = 10), and a high long-chain polyunsaturated fatty acid diet group (Unsat; n = 9). The rats were maintained on the diets for 8 wk and then euthanized. Echocardiography and tail-cuff blood pressure measurements were repeated after 4 wk on the diets.

Diets. The control diet was a commercially available normal rodent chow of which carbohydrate, protein, and fat provided 70, 20, and 10% of calories, respectively (Teklad). The carbohydrate was derived from corn starch, maltodextrin, and sucrose, which made up 45, 5, and 50% of carbohydrate calories, respectively, and the fat was derived from soybean oil and lard. The high long-chain saturated fatty acid diet provided 60% of calories from cocoa butter, and the high polyunsaturated diet was derived from safflower oil, which provided 60% of calories from fat. The fatty acid composition of the three chows was measured by gas chromatography-mass spectrometry as previously described (1) and is reported in Table 1. The palmitate concentration in the diets was 4, 17, and 12% of the total calories for the NC, Sat, and Unsat diets, respectively.

*Echocardiography.* LV function was evaluated with a Sequoia C256 system (Siemens Medica) with a 15-MHz linear array transducer as previously described (25). Briefly, rats were anesthetized with 1.2–2.0% isoflurane by mask, the chest was shaved, the animal was situated in the supine position on a warming pad, and ECG limb electrodes were placed. Two-dimensional guided M-mode, two-dimensional, and Doppler echocardiographic studies of aortic and transmural flows were performed from parasternal and foreshortened apical windows (25). All data were analyzed offline with software resident on the ultrasound system at the end of the study. LV end-diastolic and end-systolic areas were planimetered from the parasternal long-axis, and area of fractional shortening was calculated as previously described by Morgan et al. (25) and Okere et al. (26).

**Metabolic measurements.** Plasma free fatty acid and triglycerides were measured by using enzymatic spectrophotometric assays (Wako and Sigma, respectively). Blood glucose concentration was also measured by enzymatic spectrophotometric assays from perchloric acid deproteinized whole blood samples (Stanbio). Serum levels of leptin and adiponectin and plasma concentration of insulin were measured by ELISA (ALPCO Diagnostics, Salem, NH). Myocardial activities of medium-chain acyl-CoA dehydrogenase (MCAD) and citrate synthase were measured spectrophotometrically as previously described (27). Tissue triglyceride content was measured from homogenate extracts using enzymatic spectrophotometric assay of triglycerides (2). Palmitoyl ceramide (C16 ceramide) content was measured by gas chromatography with flame ionization detector using C17 ceramide as an internal standard, as previously described (36). All tissue measurements are expressed per gram wet mass.

**Histological assessments of apoptosis.** The presence of nuclear DNA fragmentation, a marker of apoptosis, was assessed in frozen LV sections with the use of ApoTag in situ fluorescein apoptosis detection kit (Oncom). The DeadEnd fluorometric terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling system measures the fragmented DNA of apoptotic cells by incorporating fluorescein-12-dUTP at 3′-OH DNA ends using the terminal deoxynucleotidyl transferase. Sections were also stained with ventricular anti-myosin

<table>
<thead>
<tr>
<th>Table 1. Fatty acid composition</th>
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<tr>
<td>Fatty Acid</td>
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<tr>
<td>16:0</td>
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<td>18:0</td>
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<td>18:1</td>
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<td>18:2</td>
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Values are expressed as percentage of total calories in chow. NC, normal chow control group; Sat, high long-chain saturated fatty acid diet group; Unsat, high long-chain polyunsaturated fatty acid group.
antibody to identify cells of cardiomyocyte origin as previously described (12, 29).

**mRNA measurements.** RNA extraction and quantitative RT-PCR were performed on frozen, powdered LV tissue using previously described methods (6, 9, 13). Specific quantitative assays were designed from rat sequences available in GenBank for expression of atrial natriuretic factor and genes that are known to be regulated by PPARα: MCAD, pyruvate dehydrogenase kinase-4 (PDK-4), and uncoupling protein-3 (UCP-3). Standard RNA was made for all assays by the T7 polymerase method (Ambion), using total RNA isolated from rat hearts. The correlation between the number of PCR cycles required for the fluorescent signal to reach a detection threshold and the amount of standard was linear over at least a 5-log range of RNA for all assays. mRNA concentration was normalized to cyclophilin, which was quantitatively measured in each sample and was not different among the experimental groups (data not shown).

**Statistical analysis.** A one-way ANOVA was used to compare mass measurements, hemodynamic function, plasma free fatty acids and triglyceride, and tissue contents of triglyceride among diet groups. A two-way ANOVA was used for the comparison of echocardiographic data among groups. All values are recorded as means ± SE. *P < 0.05 vs. NC; †P < 0.05 vs. NC and Sat.

### RESULTS

**Body and visceral adipose tissue mass.** There were no differences in body mass before or after treatment, nor was there any effect of diet on weight gain (Table 2). Food consumption was not different among groups, with the average daily consumption of 184 ± 15, 170 ± 5, and 187 ± 22 kcal for NC, Sat, and Unsat groups, respectively. The fat mass in the intrathoracic space was greater in the Sat group than the NC or Unsat group (Table 2). On the other hand, the mass of intra-abdominal and epididymal fat was lower in the Sat group than the Unsat group.

**Metabolic parameters.** Plasma free fatty acid concentration was significantly higher in the Sat group compared with the NC group, and there were no significant differences in body mass before or after treatment, nor was there any effect of diet on weight gain (Table 2). Food consumption was not different among groups, with the average daily consumption of 184 ± 15, 170 ± 5, and 187 ± 22 kcal for NC, Sat, and Unsat groups, respectively. The fat mass in the intrathoracic space was greater in the Sat group than the NC or Unsat group (Table 2). On the other hand, the mass of intra-abdominal and epididymal fat was lower in the Sat group than the Unsat group.

**Metabolic parameters.** Plasma free fatty acid concentration was significantly higher in the Sat group compared with the NC group, and there was no significant difference between the Sat and Unsat or the NC and Unsat groups (Table 2). Serum insulin levels were significantly lower in the Sat group compared with the NC group, and there was no significant difference between the Sat and Unsat or the NC and Unsat groups (Table 2). Serum leptin was significantly lower in the Sat group compared with the NC group, and plasma triglyceride level was significantly higher in the Unsat group when compared with the NC and Sat treatment groups but was significantly higher in the Sat group compared with the Unsat group (Fig. 3). Cardiac mRNA expression of atrial natriuretic factor, a marker of cardiac hypertrophy and heart failure, was similar among treatment groups (Fig. 4). Cardiac expression of the

| Table 2. Body and left ventricle mass measurements |
|-----------------------------------|---|---|
| Pretreatment body mass, g          | NC | Sat | Unsat |
| Terminal body mass, g              | 335±13 | 327±3 | 326±13 |
| Abdominal fat/body mass, g/kg      | 42.5±4.7 | 33.5±1.5 | 49.8±3.2† |
| Epididymal fat/body mass, g/kg     | 25.9±2.3 | 20.2±1.5* | 25.2±1.4 |
| Intrathoracic fat/body mass, g/kg  | 0.78±0.11 | 1.31±0.09† | 0.83±0.12 |
| LV mass, g                        | 1.01±0.03 | 0.96±0.02 | 0.99±0.04 |
| RV mass, g                        | 0.28±0.02 | 0.27±0.01 | 0.28±0.03 |
| LV mass/body mass, g/kg           | 1.80±0.05 | 1.80±0.03 | 1.80±0.04 |
| Biventricular mass index, g/kg    | 2.29±0.07 | 2.31±0.03 | 2.29±0.07 |

Values are means ± SE. LV, left ventricular; RV, right ventricular. *P < 0.05 Sat vs. Unsat; †P < 0.05 Sat vs. NC and Unsat; ‡P < 0.05 Unsat vs. Sat.

| Table 3. Plasma lipid concentrations |
|--------------------------------------|---|---|---|
| Plasma free fatty acid, mM           | NC | Sat | Unsat |
| Plasma triglyceride, mg/ml           | 1.52±0.16 | 1.31±0.12 | 0.57±0.06† |

Values are means ± SE. *P < 0.05 vs. NC; †P < 0.05 vs. NC and Sat.

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**Fig. 1.** Serum insulin and leptin concentrations under unfasted conditions after 8 wk of dietary treatment. Unsat, unsaturated fatty acid. *P < 0.05 compared with normal chow control (NC) group; †P < 0.05 compared with NC and saturated fatty acid (Sat) groups.
PPARα-regulated fatty acid oxidation enzymes, PDK-4, and UCP-3 were elevated to similar extents (2.5-fold increases) by Sat and Unsat feeding compared with the NC-fed group (Fig. 4). Sat feeding led to significant elevation in MCAD gene expression; however, the Unsat group was not different from the NC group. The activity of MCAD was not different among groups (13 ± 1, 12 ± 1, and 11 ± 1 μmol·min⁻¹·g⁻¹ in the NC, Sat, and Unsat groups, respectively), suggesting that the upregulation of MCAD mRNA was not translated into a greater amount of active protein. The activity of citrate synthase was similar among groups (164 ± 7, 181 ± 7, and 164 ± 1 μmol·min⁻¹·g⁻¹ in the NC, Sat, and Unsat groups, respectively), suggesting that there was no difference in mitochondrial content among groups.

**Cardiac function and blood pressure.** There were no significant differences among groups in the prediet echocardiograms or tail-cuff blood pressures (data not shown). Eight weeks of dietary treatment did not change arterial systolic blood pressure, heart rate, or LV pressure (Table 4). Echocardiography measurements showed similar values for all treatment groups (Table 5).

**Apoptosis.** The number of cardiomyocyte apoptotic events was higher in the Sat group compared with the Unsat group (0.28 ± 0.05 vs. 0.17 ± 0.04 apoptotic cells/1,000 nuclei; *P* < 0.05 compared with NC and Sat groups.)
DISCUSSION

The results of this study showed that the fatty acid composition of high-fat diets differentially affected PPAR-regulated gene expressions, fat distribution, and cardiomyocyte apoptosis. In addition, serum leptin and insulin levels were reduced only in the long-chain saturated fat-fed rats. On the other hand, neither high-fat diet altered cardiac mass or adversely affected cardiac function; thus 2 mo on a high-fat diet does not cause any obvious toxicity in the heart. Taken together, these findings suggest divergent effects of high-fat diets composed of saturated and unsaturated fatty acids on cardiac metabolic phenotype and apoptosis.

A commonly proposed mechanism for lipid-induced cardiomyocyte death is via the activation of caspases by ceramide, a potent proapoptotic by-product of lipid metabolism (15, 37, 38). Palmitate is the primary fatty acid moiety in cardiac ceramide (34), and thus one would expect to induce a higher rate of cardiac cell death in a diet high in palmitate compared with a diet low in palmitate but high in unsaturated fatty acid, as observed in the present investigation (Fig. 1). Ceramide is either produced via de novo synthesis from palmitate or by the hydrolysis of membrane sphingomyelin by the action of sphingomyelinase. Our results suggest that feeding a diet high in palmitate increases myocardial palmitoyl-ceramide content, whereas a high unsaturated fatty acid diet results in significantly reduced ceramide content. Moreover, the number of apoptotic events was significantly lower in the Unsat-fed rats than the Sat-fed animals. This is consistent with findings in cultured neonatal cardiomyocytes, which showed that addition of oleate to the medium prevented palmitate-induced apoptosis (14, 34). Taken together, these findings suggest that consuming a diet rich in unsaturated fatty acids may reduce loss of cardiomyocytes due to apoptosis. The present investigation did not address the lifelong effects of consuming a diet rich in unsaturated fatty acids; however, one might speculate that such a diet might prevent cardiac dysfunction and/or LV remodeling during senescence due to cumulative apoptosis. Additional studies are required to examine the more long-term effects of dietary lipid on cardiac apoptosis and function.

In the present study, we observed a similar increase in the cardiac expression of the PPAR genes PDK-4 and carnitine palmitoyltransferase 1 in both high-fat-fed groups; however, the expression of MCAD mRNA was only significantly elevated by saturated fat feeding. In addition, the activity of MCAD was not affected by high-fat feeding, nor was the activity of citrate synthase, an index of mitochondrial content. Thus the increase in MCAD mRNA did not translate into greater enzyme activity. As we previously observed with metabolic genes and proteins in dogs and rats with heart failure (20, 24), alterations in gene expression frequently do not translate into changes in protein expression or activity. The mechanism for the differential regulation of MCAD mRNA by the two diet remains to be elucidated.

The results of this study showed that the fatty acid composition of the high-fat diet differentially affected fat distribution and plasma leptin levels but had no effect on serum adiponectin concentration. Sat feeding induced greater intrathoracic adiposity and reduced epididymal and abdominal fat compared with the Unsat group, suggesting that long-chain saturated fat is not as readily stored as triglyceride in adipocytes in these regions. The reduction in serum leptin level in the Sat group and the significant positive correlation with epididymal and abdominal fat stores suggest that long-chain saturated fatty acids do not induce leptin secretion from adipose and thus

Table 5. Echocardiography results after 8 wk on diet

<table>
<thead>
<tr>
<th></th>
<th>NC</th>
<th>Sat</th>
<th>Unsat</th>
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<tr>
<td>LV end-diastolic diameter, cm</td>
<td>0.82±0.02</td>
<td>0.85±0.02</td>
<td>0.78±0.02</td>
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<tr>
<td>LV end-systolic diameter, cm</td>
<td>0.42±0.02</td>
<td>0.47±0.02</td>
<td>0.36±0.03</td>
</tr>
<tr>
<td>Area of fractional shortening, %</td>
<td>65.2±2</td>
<td>62±3</td>
<td>67±2</td>
</tr>
<tr>
<td>Velocity of circumferential shortening, s⁻¹</td>
<td>7.0±0.3</td>
<td>6.9±0.4</td>
<td>8.5±0.6</td>
</tr>
<tr>
<td>Myocardial performance index</td>
<td>0.40±0.02</td>
<td>0.43±0.02</td>
<td>0.43±0.03</td>
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<tr>
<td>Cardiac index, ml/min · kg⁻¹</td>
<td>91±5</td>
<td>110±6</td>
<td>89±5</td>
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<tr>
<td>Relative wall thickness</td>
<td>0.45±0.01</td>
<td>0.40±0.03</td>
<td>0.46±0.02</td>
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Values are means ± SE. * P < 0.05 Sat vs. NC and Unsat.
reduce circulating leptin concentration. These changes in leptin were observed in the absence of any changes in body weight. Our data suggest that fat mass may not be the only determinant of circulating leptin. Studies in isolated cardiomyocytes demonstrate that elevated leptin levels stimulate hypertrophy (41) and chronic leptin administration in vivo increases arterial blood pressure (7, 28, 33), suggesting that a dietary-induced reduction in leptin may be beneficial to the heart. Clearly, additional work is needed to elucidate the mechanism for the reduced leptin levels with the Sat diet and the effects of chronic alterations in leptin on the heart.

In summary, feeding a high-fat diet for 2 mo did not affect body mass, cardiac function, or LV mass. On the other hand, feeding a high saturated fat diet resulted in lower insulin and leptin concentrations in the plasma compared with either a low-fat diet or a high unsaturated fat diet. The high-fat diet differentially upregulated the expression of PPAR-regulated genes, but neither diet affected the activity of the mitochondrial enzymes citrate synthase or MCAD. The high unsaturated fat diet reduced cardiac ceramide content compared with the high saturated fat diet, which corresponded with a reduced frequency of cardiomyocyte apoptosis. Thus these results suggest that over a 2-mo period, high-fat diets do not adversely affect cardiac function in the absence of effects on weight gain.

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GRANTS

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REFERENCES


