Linoleate-Rich High-Fat Diet Decreases Mortality in Hypertensive Heart Failure Rats Compared With Lard and Low-Fat Diets

Adam J. Chicco, Genevieve C. Sparagna, Sylvia A. McCune, Christopher A. Johnson, Robert C. Murphy, David A. Bolden, Meredith L. Rees, Ryan T. Gardner, Russell L. Moore

Abstract—Recent studies indicate that high-fat diets may attenuate cardiac hypertrophy and contractile dysfunction in chronic hypertension. However, it is unclear whether consuming a high-fat diet improves prognosis in aged individuals with advanced hypertensive heart disease or the extent to which differences in its fatty acid composition modulate its effects in this setting. In this study, aged spontaneously hypertensive heart failure rats were administered a standard high-carbohydrate diet or high-fat diet (42% of kilocalories) supplemented with high-linoleate safflower oil or lard until death to determine their effects on disease progression and mortality. Both high-fat diets attenuated cardiac hypertrophy, left ventricular chamber dilation, and systolic dysfunction observed in rats consuming the high-carbohydrate diet. However, the lard diet significantly hastened heart failure mortality compared with the high-carbohydrate diet, whereas the linoleate diet significantly delayed mortality. Both high-fat diets elicited changes in the myocardial fatty acid profile, but neither had any effect on thromboxane excretion or blood pressure. The prosurvival effect of the linoleate diet was associated with a greater myocardial content and linoleate-enrichment of cardiolipin, an essential mitochondrial phospholipid known to be deficient in the failing heart. This study demonstrates that, despite having favorable effects on cardiac morphology and function in hypertension, a high-fat diet may accelerate or attenuate mortality in advanced hypertensive heart disease depending on its fatty acid composition. The precise mechanisms responsible for the divergent effects of the lard and linoleate-enriched diets merit further investigation but may involve diet-induced changes in the content and/or composition of cardiolipin in the heart. (Hypertension. 2008;52:549-555.)

Key Words: diet ■ heart failure ■ hypertrophy ■ mortality ■ rats
severe cardiomyopathy in Barth syndrome. Decreases in the LA enrichment of myocardial CL have also been reported in the more prevalent dilated and ischemic forms of HF in humans and in animal models of pressure-overload hypertrophy. Recently, we reported that a progressive loss of LsCL correlates closely with the development of HF in aged spontaneously hypertensive HF (SHHF) rats. Collectively, these studies indicate that LsCL deficiency is common to many forms of HF; however, no studies to date have examined its potential as a therapeutic target in the treatment of the disease. Dietary LA supplementation has been shown to effectively reverse decreases in LsCL induced by dietary LA restriction, but its effects on LsCL deficiency and prognosis in hypertensive heart disease have not been investigated previously. In the present study, we hypothesized that a diet enriched in LA would preserve cardiac LsCL levels and improve survival compared with a lard-supplemented and standard high-carbohydrate diet in aged SHHF rats with advanced hypertensive heart disease.

**Methods**

**Animal Model and Diets**

Male lean SHHF rats (McCoo/−/−) were maintained on a standard low-fat rat chow diet (Purina 5001) ad libitum until 18 months of age, when they routinely exhibit pronounced cardiac hypertrophy and marked reductions in cardiac LsCL. Animals were then divided into 3 diet groups: a standard high-carbohydrate, low-fat (Purina 5001) chow diet (CON; n=13), a high-fat diet consisting of 5001 chow with 20% (weight/weight) high-LA safflower oil (HLSO; n=16), or a high-fat diet consisting of 20% (weight/weight) lard (n=13). The Purina 5001 chow was selected as the base diet because it is well tolerated by SHHF rats throughout their lifetime and derives only 13% of total carbohydrate kilocalories from simple sugars (7% of total kilocalories). This distinction has been noted because of recent evidence that a diet rich in simple sugars (eg, 70% fructose) adversely influences mortality and cardiac function compared with complex carbohydrates in hypertensive rats. Animals were assigned to the 3 groups randomly, but care was taken to match the animals in each group to control for any inherited variations that might exist between litters. Animals were maintained on the diets ad libitum until they died or were euthanized because of overt terminal HF or severe age-related pathology.

**Characterization of terminal HF was based on presentation of a combination of external clinical signs (eg, orthopnea/dyspnea, cyanosis, piloerection, and lethargy) and internal evidence of HF (eg, ventricular dilatation and contractile dysfunction on echocardiography, atrial dilatation and thrombi, peritoneal fluid, pulmonary edema, and pleural effusion) described previously in this model.** An analysis of the precise nutrient and fatty acid compositions of the 3 diets and details regarding the determination of HF and non-HF mortality are available in the online data supplement (please see http://hyper.ahajournals.org). All of the animals were treated according to the guidelines conforming to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health and University of Colorado Animal Care and Use Committee.

**Myocardial CL, Fatty Acids, and Citrate Synthase Activity**

The CL molecular species profile was determined in left ventricular (LV) tissue homogenates by our previously published electrospray ionization mass spectrometry method. "Total CL" represents the m/z sum of the 8 most prevalent CL species detected. The global LV fatty acid profile of LV homogenates was determined by gas chromatography/mass spectroscopy methods described previously. Citrate synthase activity was determined in LV tissue to provide a comparative estimate of tissue mitochondrial density in the 3 experimental groups.

**Blood Pressure and Thromboxane Excretion**

Tail-cuff blood pressure measurements were obtained at baseline (18 months) and after 3 months on the diets (21 months) using the Gilson Duograph system in unheated isoflurane-anesthetized rats. Thromboxane A2 is a potent prohypertensive eicosanoid derived from endoperoxides synthesized by cyclooxygenase from arachidonic acid. Because arachidonic acid is primarily derived from LA consumed in the diet, renal thromboxane excretion was determined in duplicate by an enzyme immunoassay (Cayman Chemical) in urine collected from rats placed in metabolic cages for 24 hours after 3 months on each of the experimental diets.

**Echocardiography**

Transthoracic echocardiography was performed on rats at baseline (18 months), after 3 months on the diets (21 months), and immediately before sacrifice (HF) under inhaled isoflurane anesthesia (5% initial and 2% maintenance) using a 12-MHz pediatric transducer connected to a Hewlett Packard Sonos 5500 Ultrasound, as described previously.

**Statistical Analyses**

All of the data are presented as group means±SEs. Cumulative survival probability was plotted on a Kaplan–Meier curve with pairwise comparisons of diets using the log-rank statistic. Group means for all of the data were compared using ANOVAs with Tukey tests post hoc when appropriate. Pearson correlation analyses were performed to determine the relationship between CL data and age of terminal HF. Statistical significance was established at P<0.05 for all of the analyses.

**Results**

**Animal Characteristics and Incidence of Terminal HF**

As reported recently in a survival study using aged SHHF rats, some of the animals in this study died or developed age-related pathology (eg, mammary and/or pituitary tumors) that necessitated euthanasia before manifestation of terminal HF (3 in the CON and 5 in the HLSO groups). These animals exhibited significantly lower heart and lung weights and lacked other classic internal features associated with terminal HF (eg, pulmonary edema and pleural effusion, peritoneal ascites, ventricular dilatation, and contractile dysfunction) and were, therefore, excluded from all of the subsequent analyses. Data from these animals excluded because of "non-HF mortality" are available in the online data supplement. The mean age of euthanized animals because of non-HF pathology was similar between the CON (22.8±0.6 months) and HLSO (23.2±1.2 months) groups. The absence of non-HF mortality in the lard group may be explained by the earlier onset of terminal HF in this group compared with the CON and HLSO groups (Table 1 and Figure 1), resulting in death before the development of severe age-related pathology. Body and tissue weights of animals exhibiting terminal HF at the time of sacrifice are presented in Table 1. A significant loss of body weight occurred in all of the groups during the experimental period, with no significant differences among the 3 groups. Absolute and relative (normalized to brain weight) heart weights were significantly lower in animals fed the HLSO (P<0.05) and lard diets (P<0.05), with no difference between the 2 high-fat diets. There were no significant differences among the groups on any other morphological parameters.
Mortality data and Final Animal Characteristics

<table>
<thead>
<tr>
<th>Data</th>
<th>CON</th>
<th>Lard</th>
<th>HLSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terminal HF, incidence, n/No. (%)</td>
<td>10/13 (77)</td>
<td>13/13 (100)</td>
<td>11/16 (68)</td>
</tr>
<tr>
<td>HF mortality, age</td>
<td>23.5±0.4</td>
<td>22.2±1.5</td>
<td>25.0±0.6†</td>
</tr>
<tr>
<td>All-cause mortality, age</td>
<td>23.3±0.3</td>
<td>22.2±1.5</td>
<td>24.6±0.5†</td>
</tr>
</tbody>
</table>

Mortality data

Cumulative Mortality

Mean age of mortality resulting from HF and all causes was significantly greater in animals fed the HLSO diet compared with the CON and lard diets (Table 1). Figure 1 illustrates the effect of the diets on cumulative mortality resulting from HF and all causes. The HLSO diet significantly increased HF survival probability compared with the CON and lard diets (P<0.001), whereas the lard diet decreased HF survival probability (P<0.05). Cumulative all-cause mortality rate was also significantly delayed in HLSO compared with the lard (P<0.01) and CON (P<0.05) groups.

Myocardial CL and Fatty Acid Profile

In the healthy rat myocardium, L4CL represents ∼77% of total CL species,15 which is similar to levels reported previously in 2-month-old (nonfailing) SHHF rats.17 In the present study, terminal HF (CON) was associated with a marked loss of L4CL (42% of total CL), as reported previously17,20 (Figure 2A). The HLSO diet markedly increased L4CL in the CL pool compared with CON and lard (P<0.001), restoring levels to 77% of total CL, whereas the lard diet had a statistically insignificant depressive effect (38% of total CL). HLSO also significantly increased total CL content compared with CON and lard (P<0.05), whereas the lard diet elicited a slight decrease in CL content (P<0.05 versus CON; Figure 2B). Although a detailed assessment of tissue mitochondrial content was not performed, myocardial citrate synthase activity, an exclusive marker of the mitochondrial matrix and indirect index of mitochondrial density, was similar among CON (84±15 nmol·min⁻¹·mg protein⁻¹), lard (80±10 nmol·min⁻¹·mg protein⁻¹), and HLSO (78±10 nmol·min⁻¹·mg protein⁻¹), indicating that the observed changes in CL content were not likely because of changes in tissue mitochondrial content. Both L4CL and total CL levels correlated positively with age of mortality (Figure 2C and 2D), indicating that greater L4CL and total CL levels were directly associated with increased survival.

The effects of the high-fat diets on the global myocardial fatty acid profile from rats in terminal HF are presented in Table 2. The HLSO diet resulted in significantly greater levels of LA and eicosapentaenoic acid (20:5n3), whereas α-linolenic (18:3n3), arachidonic (20:4n6), and docosahexaenoic acid (22:6n3) levels were lower compared with the CON and lard groups. Palmitic acid (16:0) content was significantly greater in the lard versus the CON and HLSO groups, whereas LA was significantly lower (P<0.05).

Blood Pressure and Thromboxane Excretion

There were no significant differences in systolic blood pressure among the CON (209±5 mm Hg), lard (212±3 mm Hg), or HLSO (210±4 mm Hg) groups at baseline (before beginning the diets) or after 3 months on the diets (203±4, 214±3, and 209±4 mm Hg, respectively). Thromboxane excretion was not significantly different in the lard (111±50% of CON) or HLSO (104±15% of CON) groups compared with the CON group (100±21%) after 3 months on the diets.

Echocardiography

Echocardiography data obtained at baseline, after 3 months on the diets, and in all of the surviving animals in terminal HF...
are presented in Figure 3. No significant differences on any measures existed between the groups at baseline. Significant increases in LV internal diameter in diastole and systole were evident in the CON animals at 21 months and HF ($P<0.05$), consistent with the development of dilated systolic HF. The lard and HLSO diets prevented significant increases in LV internal diameter in diastole at both time points. No changes in LV posterior wall thickness in diastole were observed in CON animals throughout the study, although the lard and HLSO diets elicited significant increases relative to baseline values at both time points. Lard and HLSO diets attenuated the decline in fractional shortening seen in the CON group at both time points. No significant differences were observed between the lard and HLSO groups on any echocardiography measures during the course of the study, indicating a similar effect of both high-fat diets on cardiac function and morphology during the pathogenesis of HF.

**Discussion**

The primary finding of this study is that 2 high-fat diets administered to animals with advanced hypertensive heart disease elicited opposite effects on HF mortality despite having similar effects on cardiac morphology and function. Our data corroborate previous evidence that high-fat diets attenuate hypertension-induced cardiac hypertrophy and systolic dysfunction\(^9,^{10}\) but demonstrate that the fatty acid composition of a high-fat diet is a critical determinant of its effect on HF mortality. The mechanisms by which the lard and HLSO diets elicited such diametrically opposed opposite effects on survival are not entirely clear from this study, but our data demonstrate that they are independent of changes in systolic blood pressure and are closely associated with changes in the content and composition of myocardial CL.

Okere et al\(^9,^{10}\) demonstrated that administration of a high-fat diet (60% of kilocalories consisting primarily of stearate, 18:0) attenuates the cardiac hypertrophy, remodeling, and contractile dysfunction associated with high-salt feeding in Dahl salt-sensitive rats without any reduction in systolic blood pressure. We observed nearly identical effects with the lard and HLSO diets in aged SHHF rats in the present study, indicating that high-fat diets of different fatty acid compositions appear have similar effects on cardiac morphology and function in the presence of chronic hypertension independent of any modulation of cardiac afterload. The mechanisms by which high-fat diets elicit these effects were not examined in the

**Table 2. Effect of Diets on the Global LV Fatty Acid Profile**

<table>
<thead>
<tr>
<th>Fatty Acid (% Total)</th>
<th>CON</th>
<th>Lard</th>
<th>HLSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic (16:0)</td>
<td>9.6±0.4</td>
<td>11.6±0.2(†)</td>
<td>9.7±0.3</td>
</tr>
<tr>
<td>Stearic (18:0)</td>
<td>17.6±1.3</td>
<td>18.8±0.7</td>
<td>19.5±0.4</td>
</tr>
<tr>
<td>Oleic (18:1n9)</td>
<td>3.2±0.3</td>
<td>3.7±0.3</td>
<td>3.1±0.1</td>
</tr>
<tr>
<td>Linoleic (18:2n6)</td>
<td>17.2±0.9</td>
<td>12.5±0.8(§)</td>
<td>26.6±1.2(§)</td>
</tr>
<tr>
<td>α-Linolenic (α 18:3n3)</td>
<td>0.13±0.01</td>
<td>0.09±0.01</td>
<td>0.06±0.01(§)</td>
</tr>
<tr>
<td>Arachidonic (20:4n6)</td>
<td>40.1±1.9</td>
<td>43.4±1.2</td>
<td>35.1±1.3(†)</td>
</tr>
<tr>
<td>EPA (20:5n3)</td>
<td>0.37±0.04</td>
<td>0.24±0.01</td>
<td>0.66±0.05(§)</td>
</tr>
<tr>
<td>DHA (22:6n3)</td>
<td>11.6±1.0</td>
<td>9.5±1.0</td>
<td>5.3±0.9(†)</td>
</tr>
</tbody>
</table>

DHA indicates docosahexaenoic acid; EPA, eicosapentaenoic acid.

\(†P<0.05\) vs CON.
\(‡P<0.01\) vs CON. \(\daggerP<0.05\) vs LA or lard.
\(P<0.01\) vs LA or lard.

\(§P<0.01\) vs LA or lard.
present study, but previous studies indicate that a suppression of insulin-mediated hypertrophic signaling and/or activation of the peroxisome proliferator-activated receptor-α may be involved (recently reviewed by Sharma et al). The primary aims of this study were to examine the effects of an LA-enriched diet on L₄CL and HF mortality based on evidence that cardiac L₄CL deficiency is sufficient to cause lethal cardiomyopathy in Barth syndrome and is associated with common forms of HF in humans and animal models. Dietary LA supplementation has been shown previously to reverse experimentally induced L₄CL deficiency, but the present study is the first to demonstrate that an LA-enriched diet effectively preserves L₄CL during the pathogenesis of HF. The mechanism by which the HLSO and lard diets altered L₄CL levels was not directly examined in this study, but biosynthesis of L₄CL is achieved by ≥2 CL remodeling pathways that require LA as a substrate (esterified to coenzyme A or to glycerol in diacyl phospholipids, such as phosphatidylcholine). The extent to which dysfunction of CL remodeling pathways is responsible for L₄CL deficiency in the failing heart is presently unknown, but the >50% increase in myocardial LA content induced by the HLSO diet suggests that L₄CL levels may have been restored simply by mass action, overwhelming any deficiencies in CL remodeling capacity. However, at this point we cannot exclude the possibility that the HLSO and lard diets directly modulated CL biosynthesis and/or degradation pathways. It has been demonstrated previously that palmitate (elevated in hearts from rats fed the lard diet herein) decreases CL content in cardiomyocytes by interfering with the biosynthesis of CL when doubly esterified to phosphatidylglycerol. In the present study, the lard diet significantly decreased myocardial LA content compared with the CON diet despite having a greater LA content than the CON diet, suggesting that it may have altered CL synthesis, remodeling, and/or degradation processes. How LA and other dietary fatty acids directly modulate CL biosynthesis and remodeling requires further investigation.

The significant correlation of survival with myocardial L₄CL and CL contents suggests that the diets may have affected mortality by modulating the content and/or composition of CL. Several proteins and processes involved in mitochondrial energy metabolism are known to require CL for optimal function, and impaired mitochondrial function likely contributes to the progression of HF. Furthermore, reductions in CL content can trigger apoptotic signaling in cardiomyocytes, which may also accelerate HF progression. Therefore, it is plausible that increasing myocardial levels of CL in its optimal L₄ configuration may delay terminal HF by attenuating mitochondrial dysfunction and apoptosis during the advancing stages of the disease. Now that the survival benefit and CL restorative effects of the HLSO diet have been established, future investigations will focus on elucidating the cellular manifestations of this intervention during the various stages of hypertensive heart disease.

Although the pathological consequences of myocardial CL deficiency have been well established, the HLSO and lard diets may have modulated HF mortality by other mechanisms. In addition to being associated with a proatherogenic serum lipid profile, consumption of saturated and trans-fats present in the lard diet may increase production of proinflammatory cytokines, such as tumor necrosis factor-α, in HF patients. Moreover, saturated fatty acids, particularly palmitate, are known to result in ceramide accumulation and apoptosis in cardiomyocytes; however, this has been re-
ported recently after a saturated fat–rich diet independent of any adverse effects on cardiac function.43

Consumption of PUFA s is generally associated with reduced cardiovascular risk,44 but benefits have been ascribed primarily to the omega-3 PUFA s, principally, docosahexaenoic acid (22:6n3) and eicosapentaenoic acid (20:5n3), because of their putative antiarrhythmic, antihypertensive, and antiinflammatory effects.45 In fact, some groups recommend that omega-6 PUFA intake be limited relative to n-3 PUFA s,46 given evidence that LA may limit docosahexaenoic acid and eicosapentaenoic acid synthesis from α-linolenic acid (18:3n3),47 or promote increases in arachidonic acid (20:4n6) and its proinflammatory and prohypertensive metabolites. Interestingly, the HLSO diet in the present study improved survival despite decreasing myocardial

per reception of their putative antiarrhythmic, antihypertensive, and proinflammatory and prohypertensive metabolites. Absorbed after a saturated fat–rich diet independent of any adverse effects on cardiac function.43

Limitations of the Study
As stated above, the primary aim of this study was to determine the effect of the selected diets on the cardiac CL profile and mortality in the SHHF rat model. Although this investigation has provided novel insight into the effects of dietary fatty acid composition on long-term prognosis in hypertensive heart disease, there are limitations associated with the survival study design that warrant further comment. In particular, preparation of high-quality mitochondria for functional assessments was not feasible in this study given the inconsistent and unpredictable timing of animal death or sacrifice. However, it is also plausible that any subcellular effects elicted by the diets that could have influenced mortality might have occurred early during the course of disease progression and would no longer be evident when animals progressed to terminal HF. Therefore, the extent to which the selected diets elicit such changes during the early and late stages of disease progression requires further study and is currently under investigation in our laboratories. Finally, the decision to sacrifice animals and determination of HF versus non-HF mortality was based on a well-documented series of clinical HF symptoms, tissue morphology, and echocardiography indices that coincide with classic histological (e.g., fibrosis) and biochemical markers (myosin heavy chain isoymes and atrial natriuretic peptide) of HF established previously in this model.24–26 However, no additional analyses were performed to further characterize the precise cause of death or the extent of disease progression at the time of sacrifice.

Perspectives
Although consumption of a low-fat diet is currently recommended for optimal cardiovascular health,6 recent studies indicate that a high-fat diet may attenuate the progressive cardiac hypertrophy and contractile dysfunction associated with chronic hypertension without altering systolic blood pressure.4,9,10 The present study corroborates these findings in an established model of senescent hypertensive heart disease but demonstrates that a high-fat diet may increase or decrease HF mortality depending on its fatty acid composition. The prosurvival effect of the HLSO diet may result in part from a preservation of a favorable CL profile in the heart, but further studies are needed to elucidate the physiological consequences of this effect. Moreover, it will be important to examine the many other effects that LA and/or HLSO may have on cardiovascular parameters in patients with advanced cardiac disease before considering the clinical feasibility of this intervention. It is worth noting, however, that serum and dietary intake of LA have been associated previously with reduced cardiovascular disease incidence and mortality in humans.44,45 Therefore, determining how LA and other dietary fatty acids modulate cardiac health and disease clearly merits further investigation.

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Disclosures
None.

References


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