Low Carbohydrate/High-Fat Diet Attenuates Cardiac Hypertrophy, Remodeling, and Altered Gene Expression in Hypertension

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Abstract—The effects of dietary fat intake on the development of left ventricular hypertrophy and accompanying structural and molecular remodeling in response to hypertension are not understood. The present study compared the effects of a high-fat versus a low-fat diet on development of left ventricular hypertrophy, remodeling, contractile dysfunction, and induction of molecular markers of hypertrophy (ie, expression of mRNA for atrial natriuretic factor and myosin heavy chain β). Dahl salt-sensitive rats were fed either a low-fat (10% of total energy from fat) or a high-fat (60% of total energy from fat) diet on either low-salt or high-salt (6% NaCl) chow for 12 weeks. Hearts were analyzed for mRNA markers of ventricular remodeling and activities of the mitochondrial enzymes citrate synthase and medium chain acyl-coenzyme A dehydrogenase. Similar levels of hypertension were achieved with high-salt feeding in both diet groups (systolic pressure of ≈190 mm Hg). In hypertensive rats fed low-fat chow, left ventricular mass, myocyte cross-sectional area, and end-diastolic volume were increased, and ejection fraction was decreased; however, these effects were not observed with the high-fat diet. Hypertensive animals on low-carbohydrate diet had increased atrial natriuretic factor mRNA, myosin heavy chain isoform switching (α to β), and decreased activity of citrate synthase and medium chain acyl-coenzyme A dehydrogenase, which were all attenuated by high-fat feeding. In conclusion, increased dietary lipid intake can reduce cardiac growth, left ventricular remodeling, contractile dysfunction, and alterations in gene expression in response to hypertension. (Hypertension. 2006;48:1116-1123.)

Key Words: cardiac ■ heart ■ fatty acid ■ lipid ■ mitochondria

Hypertension is a leading cause of cardiac mortality and morbidity and frequently leads to pathological left ventricular (LV) hypertrophy (LVH), contractile dysfunction, and heart failure. Current dietary guidelines recommend a low-fat/high-carbohydrate diet for patients with hypertension. However, little is known about the effects of dietary fat intake on the development of LVH in response to hypertension and the accompanying structural and molecular remodeling observed with chronic blood pressure elevation. The development of LVH in response to short-term hypertension is affected by the fat and carbohydrate composition of the diet, because hypertensive rats fed a high-fat diet had reduced LVH and improved LV systolic function compared with rats fed a high-carbohydrate diet despite a similar systolic blood pressure. It is not clear whether this effect is beneficial in the long term, because the lack of compensatory hypertrophy in response to greater wall stress could accelerate LV remodeling and progression to heart failure.

The expression of genes involved in fluid regulation, cardiac contractile function, and energy metabolism are altered in response to both pressure overload and dietary fat intake. The normal heart primarily relies on the oxidation of fatty acids in the mitochondria to provide the energy for contractile power generation. However, with advanced LVH there is a down-regulation of key enzymes in the fatty acid oxidation pathway and an increase in the relative contribution of carbohydrate to cardiac energy metabolism. Expression of genes encoding fatty acid oxidation enzymes is regulated by the activity of the peroxisome proliferator activator receptors (PPARs) α and β/δ, which are activated by consumption of a high-fat diet but can be suppressed in LVH and heart failure. It is not known whether downregulation of fatty acid oxidation enzymes in LVH is prevented by consuming a high-fat diet. In addition, it is not clear whether the antihypertrophic effect of a high-fat diet in hypertensive rats is because of greater mitochondrial oxidation of fatty acids or rather is because of extramitochondrial effects (eg, stimulation of PPARs).
The present investigation evaluated the link between dietary fat intake and the development of LVH, LV remodeling, contractile dysfunction, and molecular markers of altered cardiac metabolism and function in the setting of chronic hypertension. Studies were performed in the well-established Dahl salt-sensitive (DSS) rat model of hypertension, LVH, and remodeling. Hypertension was maintained for sufficient duration to induce LVH, remodeling, and impaired systolic function. In addition, the effects of diet on established molecular markers of LVH (mRNA expression for atrial natriuretic factor [ANF] and myosin heavy chain [MHC] isoform switching from MHC-α to MHC-β) were evaluated. In subgroups of hypertensive animals, the effects of mitochondrial fatty acid metabolism were assessed by blocking the transport of fatty acyl units across the inner mitochondrial membrane by administering the carnitine palmitoyl transferase-I (CPT-I) inhibitor oxfenicine.

### Methods

#### Animal Model

All of the measurements were performed with the investigator blinded to treatment. The animal protocol was conducted according to the Guideline for the Care and Use of Laboratory Animals (National Institutes of Health publication No. 85-23) and was approved by the Institutional Animal Care and Use Committee of the Case Western Reserve University.

#### Experimental Design

Initial pretreatment measurements were made for systolic arterial blood pressure and body mass. Echocardiographic assessment of the LV systolic function and dimensions was performed at 4, 7, and 12 weeks of treatment, and measurement of systolic arterial pressures was repeated at 6 and 11 weeks using the tail cuff method as described previously. The rats were randomized into 4 groups: a low-fat/low-salt (LF/LS, n = 10), a low-fat/high-salt (LF/HS, n = 10), a high-fat/low-salt (HF/LS, n = 10), and a high-fat/high-salt (HF/HS, n = 10) group. To assess the mitochondrial and extramitochondrial effects of a high-fat diet, 2 additional groups on high salt from each diet were treated with the CPT-I inhibitor oxfenicine (4-hydroxy-2,3-dihydro-1H-phenylglycine [Oxf]: LF/HS+Oxf (n = 11) and HF/HS+Oxf (n = 10). After 12 weeks of treatment, overnight fasted animals were anesthetized with isoflurane, LV pressure was measured, blood was drawn, and the heart was harvested for biochemical analysis (details provided in an online supplement available at http://hyper.ahajournals.org).

#### Diets

All of the diets used in the study were obtained from Research Diets Inc and were formulated as described previously.

#### Echocardiography and Terminal Hemodynamic Measurements

Echocardiographic measurements and determination of LV pressure were performed as described previously (see online supplement).

#### Metabolic Measurements

Plasma was analyzed for free fatty acids, triglycerides, and hormones (insulin and leptin) as described previously (please see online supplement). LV tissue was analyzed for the concentration of triglyceride and C16-ceramide, apoptosis, Oil Red-O staining, mRNA expression (quantitative RT-PCR of ANF, MHC-α/β, citrate synthase, medium chain acyl-CoA dehydrogenase [MCAD], and CD36; Table I, available online), citrate synthase and MCAD activities, and Western blot analysis for PPARα and the retinoid X receptor α (RXRα) proteins.

#### Statistical Analysis

All of the experimental data from the study were compared using either a 2-way or 3-way ANOVA. Three-way ANOVAs were used for data comparing diet versus treatment versus time. Two way ANOVAs were either 2×2 comparing diet (low-fat or high-fat) versus treatment (no salt or salt) or a 2×2×3 design comparing diet (low-fat or high fat) versus treatment (no salt, salt, or salt+oxfenicine). All of the posthoc comparisons were Bonferroni corrections for multiple comparisons. All of the values are recorded as mean±SEM, and a P < 0.05 level of significance was used.

#### Results

**Blood Pressure**

There were no significant differences in arterial blood pressure before initiation of treatment (Figure 1). Salt feeding increased systolic blood pressure in all of the groups and was not further elevated from 6 to 11 weeks on either diet. Systolic pressure was not different among salt-fed groups at 11 weeks but at 6 weeks was slightly but significantly lower in the HF/HS group compared with the LF/HS group. HF/LS feeding caused a modest but significant elevation in blood pressure compared with LF/LS (at 11 weeks) but was significantly lower than the salt-fed groups (Figure 1). Treatment with oxfenicine had no effect on blood pressure (data not shown). All of the treatment groups fed low-salt diets and those in the HF/HS and HF/HS+Oxf survived the 12 weeks of treatment. However, 20% of the LF/HS group (2 of 10) and 36% of the LF/HS+Oxf (4 of 11) group died before the end of the study.

**Histology**

Cardiomyocyte cross-sectional area was increased in the LF/HS group compared with the LF/LS and HF/HS groups (Figure 2). Hypertension and diet did not affect capillary density, interstitial fibrosis, or cardiomyocyte apoptosis (Table II), consistent with the lack of progression to overt heart failure.

**Body and LV Mass**

The change in body mass was not different among groups when expressed in absolute terms (Table III) or as a percentage of the initial body mass (50±3%, 40±5%, 43±2%, and
48±3% for the LF/LS, LF/HS, HF/LS, or HF/HS groups, respectively). In the LF/HS group, there was a progressive increase in the LV mass/body mass ratio, as measured by echocardiography, but no progressive increase in any of the other groups (Figure 3). After 12 weeks of treatment, the gravimetrically determined LV mass was also greater in the hypertensive low-fat diet groups compared with the normotensive LF group and the hypertensive high-fat fed group. LV mass was not different between the HF-treated groups (Figure 2; Table IV). The difference between the LF/HS and HF/HS groups persisted whether LV mass was expressed raw or normalized to either body mass or tibia length. High-fat feeding with low salt caused a slight but significant elevation in LV mass when compared with LF/LS (Table IV), which corresponded with the modest increase in blood pressure (Figures 1 and 4). On the other hand, there was no difference in LV mass or myocyte cross-sectional area between the HF/HS and either the HF/LS or LF/LS group despite the dramatic difference in systolic blood pressure (Figures 1 to 4). Right ventricular mass was not different among groups, and treatment with oxfenicine had no additional effects on LV, RV, or gain in body mass (Tables III and IV).

Cardiac Dimensions and Performance
After 12 weeks of treatment there was significant LV remodeling and systolic dysfunction with hypertension on the low-fat diet compared with the normotensive LF/LS group (Figure 5). The hypertensive animals on the high-fat diet exhibited none of these abnormalities in LV volumes or function (Figure 5), with no significant differences between the HF/HS and HF/LS or between the HF/LS and LF/LS groups. LV peak positive and negative first derivative of LV pressure (dP/dt) were increased with high-fat feeding in the nonhypertensive HF/LS group compared with the LF/LS group but not in the hypertensive HF/HS group. Treatment with oxfenicine did not affect any of these parameters. Taken together, consuming a high-fat diet prevented LV remodeling and systolic dysfunction in hypertensive animals.

mRNA Expressions for ANF and MHC
ANF mRNA in the LV was increased 11.7-fold in the hypertensive low-fat–fed animals compared with the LF/LS group, and this increase was significantly lower in the HF/HS group (4.7-fold; Figure 6; Table V). There was a 43% decrease in MHC-α isoform and a 365% increase in MHC-β mRNA expression in the LF/HS group compared with the LF/LS group (Table V) and an increase in the ratio of MHC-β/MHC-α (Figure 5). High-fat feeding partially prevented isoform switching with hypertension, as seen in the 12% decrease in MHC-α and a 165% rise in MHC-β in the HF/HS group. High-fat feeding in the absence of hypertension caused a 39% decrease in MHC-α and a 172% rise in MHC-β mRNA expressions. Total MHC expression was not significantly different among groups (Table V). Thus, a high-fat diet partially prevented the well-established upregu-
lation in ANF mRNA expression and MHC isoform switching that occurs in response to chronic pressure overload-induced LVH.

**Metabolic Results**

As shown previously, high-fat feeding reduced fasting plasma triglyceride concentration irrespective of hypertension or fatty acid oxidation inhibition with oxfenicine (Table VI). Fasting plasma free fatty acid concentration was similar among groups except for significantly lower values in the HF/HS-fed group compared with the LF/LS group. Glucose was slightly but significantly lower with hypertension in both diet groups. Fasting plasma insulin concentration was similar among groups except for a significant decrease in the LF/HS group compared with the LF/LS group. Oxfenicine treatment did not affect any of these parameters. LV tissue triglyceride content was similar among groups except for a significant decrease in the HF/HS group compared with the LF/LS group. Serum leptin concentration was decreased by hypertension to the same extent irrespective of diet group (Table VI). Oxfenicine treatment did not affect any of these parameters. LV tissue triglyceride content was similar among groups except for a significant decrease in the LF/HS group compared with the LF/LS group (Table VI). Oil Red O staining for cardiac lipids was increased by high-fat feeding, and tissue C-16 ceramide was significantly increased only by inhibition of CPT-I with oxfenicine in both diet groups (Table VI).

As expected, the cardiac mRNA expression and activity for the mitochondrial fatty acid oxidation enzyme MCAD were reduced in the hypertensive rats with LVH fed a low-fat diet compared with nonhypertensive animals on low-fat chow (Figure 7; Table VII). On the other hand, there was no significant downregulation of MCAD mRNA in the hypertensive rats fed the high-fat chow (Figure 7). The high-fat diet...
with low salt caused a significant increase in MCAD mRNA but not activity, as observed previously. Previous studies in the spontaneously hypertensive rat demonstrated that this strain has reduced levels of the fatty acid transporter CD36 and therefore, we studied whether CD36 mRNA levels were reduced by hypertension or effected by diet. There was not a decrease in CD36 mRNA with hypertension, but rather a 40% increase in the LF/HS-fed rats compared with the LF/LS group ($P<0.05$; Table VII). Treatment with the CPT-I inhibitor oxfenicine reduced the mRNA levels in the HF/HS group compared with the HF/LS group ($P<0.05$; Table VII) but had no effect in low-fat–fed animals. There were no differences among groups in the mRNA levels for citrate synthase or mCPT-I; however, the activity of citrate synthase was reduced by hypertension in the LF/HS group but not in the HF/HS group (Table VII). The expression of MCAD and CPT-I in the heart is regulated by the activity of the RXRα/PPARα heterodimer. Therefore, we measured cardiac levels of these proteins by Western blot. Protein levels of RXRα were reduced by 36% in the hypertensive low-fat–fed rats compared with LF/LS-fed animals ($P<0.05$; Figure 8) with no other difference among groups. PPARα protein levels were not downregulated by hypertension; however, there was a significant 2.5-fold increase in expression in the HF/LS group compared with LF/LS and HF/HS groups (Figure 8).

**Discussion**

The novel findings of this study are that a high-fat diet attenuated the hypertension-induced increase in LV mass, cardiomyocyte hypertrophy, LV chamber remodeling, systolic dysfunction, and induction of molecular markers of cardiac hypertrophy and dysfunction. In addition, a high-fat diet prevented the decrease in activity of the key mitochondrial enzymes MCAD and citrate synthase observed in animals on a low-fat diet with similar levels of hypertension. Thus, cardiac gene expression, LVH, systolic function, and chamber remodeling in hypertension can be affected by dietary macronutrient composition.

Results of the present investigation add further support to the concept that LVH is detrimental and that the heart is better off if LVH can be prevented when the heart is subjected to pressure overload. Clinical and animal studies suggest that the initial hypertrophic response to pressure overload is a necessary adaptation that serves to normalize wall stress. However, concentric LVH frequently precedes chamber dilation and the development of heart failure. Studies in transgenic mice with a blunted hypertrophic response to pressure overload demonstrate that preventing LVH at the expense of a high wall stress improves long-term function and prevents progression to heart failure. The present study illustrates that the reduced LVH with a high-fat diet was accompanied by prevention of LV remodeling and systolic dysfunction, consistent with the concept that reducing the initial hypertrophic response is key for the prevention of LV remodeling and heart failure in response to pressure overload.

The interactions among fat and carbohydrate intake, salt intake, hypertension, and cardiac size and function are complex and difficult to decipher in vivo. The near-identical LV mass in rats fed either a low-salt or high-salt diet with high fat occurred despite gross differences in blood pressure (Figure 2), suggesting interaction between salt and fat intake. In contrast, the dramatic upregulation of PPARα protein content in high-fat fed animals was reduced when salt was added to the chow (Figure 8), implying that hypertension and/or sodium block the effects of a high-fat diet on PPARα content. Future studies should investigate the mechanism(s) behind this interaction through induction of pressure overload using aortic banding and dietary manipulations in animals without genetic sodium intolerance.

The reduced LVH with a high-fat/low-carbohydrate diet could be because of less insulin stimulation of cardiomyocyte growth, as suggested by studies in transgenic mice and clinical studies that observed a positive relationship between plasma insulin and LVH. High-fat feeding may lower plasma insulin concentration and blunt insulin stimulation of cardiomyocyte growth. Dietary intake of carbohydrates, particularly sugars, determines the exposure of the heart to insulin and insulin-like growth factor. Stimulation of cardiomyocyte insulin receptors activates phosphoinositol-3 kinase and phosphorylation and activation of Akt. Akt then activates hypertrophic growth through stimulation of protein
synthesis and inhibition of protein breakdown. Cardiac insulin receptor knockout mice have smaller hearts and reduced activation of Akt and downstream targets and do not demonstrate the normal activation of Akt in response to feeding a standard high-carbohydrate chow.21,22 Long-term administration of the high-fat chow used in the present investigation to normal rats resulted in a 70% reduction in plasma insulin in the fed state compared with rats fed the low-fat chow.8 This suggests the possibility that plasma insulin was lower over the course of the day with the high-fat chow, resulting in less stimulation of cardiac insulin signaling and hypertrophic growth. Plasma samples in the present study were taken in the fasting state to avoid the effects of meal absorption on cardiac function and metabolite levels; however, future studies should assess plasma insulin levels and activation of the insulin signaling/hypertrophic pathways under fed conditions.

The present study and previous work26 found normal levels of PPARs protein in the hypertrophied LV in the DSS rat. Similarly, the pronounced cardiomyocyte hypertrophy that develops with pacing-induced heart failure in dogs reduces the mRNA for PPARα13 but does not change protein expression of PPARα.27 Kanda et al26 found that the PPARα/RXRα complex is decreased in nuclear extracts from DSS rats with LVH, illustrating the importance of localization of the PPARα/RXRα heterodimer to the nucleus as shown in previous studies.7,28,29 We observed a striking increase in PPARα protein levels in the HF/HS group compared with the LF/HS group despite similar levels in the LF/LS and LF/HS groups (Figure 8), suggesting that the rate of PPARα protein synthesis is reduced and/or the rate of degradation is accelerated in response to hypertension in the HF/HS group compared with the HF/LS group. Additional studies are required to elucidate the mechanism(s) responsible for these effects and to assess the extent of nuclear localization in LVH.

Chronic alterations in myocardial fatty acid metabolism or activation of PPARα can affect cardiac mass and/or contractile function. In normotensive animals, interventions that cause the heart to increase fat use in transgenic mice (eg, overexpression of PPARα,30 fatty acyl-coenzyme A synthase,31 fatty acid transport protein-1,32 or lipoprotein lipase33) result in LVH and impaired contractile function. Modest LVH without contractile dysfunction is observed with long-term administration of a PPARα agonist.34,35 Feeding a high-fat diet to normotensive Wistar rats increased the mRNA for PPARα-regulated genes but does not cause LVH.8 The hypertensive animals in the present study consuming a high-fat diet displayed increased PPAR stimulation (as seen in the increased mRNA for MCAD) and higher MCAD activity compared with low-fat–fed animals. This effect was seen, although animals were euthanized after an overnight fast, which dampens changes in cardiac mRNA for PPARα-regulated genes.36 It is possible that stimulation of PPARα during the development of pressure overload–induced hypertrophy prevents deterioration of mitochondrial function and development of LVH, remodeling, and contractile dysfunction.

The high-fat–treated rats on low-salt diet develop significant hypertrophy; however, it is not clear whether the modest degree of hypertension that developed in this group was responsible for the cardiac hypertrophy. Perhaps this modest but significant LVH that developed was not maladaptive, but rather conferred a protective effect that may be analogous to the exercised heart, compared with the type of LVH that develops in response to chronic hypertension. The large increase in PPARα protein content with the low-salt/high-fat diet suggests a unique adaptation in this group compared with all of the other treatments.

Limitations of the Study

As noted above, the contribution of simple sugar to the carbohydrate component of a low-fat diet could affect cardiomyocyte growth and function; thus, it is difficult to separate to effects of elevated dietary lipid from the elimination of simple sugar in the present investigation. A link between a diet high in simple sugar and ischemic heart disease was suggested in the 1960s and 1970s37,38; however, the relationship with LVH has not been examined, particularly in the setting of hypertension. The carbohydrate component of the low-fat chow in the present study was 50% sucrose; thus, 35% of the total energy was from sugar, suggesting the possibility that the benefit of the high-fat diet may be because of the reduction in sugar consumption. Future studies should compare diets high in either simple sugar or complex carbohydrate in hypertension.

It is important to note that the findings of this study may be limited to the experimental model and conditions that were used. Animals were not maintained for sufficient duration to fully assess effects on progression to heart failure or mortality. The DSS rat develops heart failure after ~12 weeks on an LF/HS diet10,11; thus, future studies should extend treatment with a high-fat diet and assess the effects on the progression to heart failure and mortality. In addition, future studies should use sodium-independent models of pressure overload, such as aortic constriction, because the observations of the present study could be unique to the DSS rat and high-sodium intake. This would allow the use of transgenic mice with altered insulin signaling pathways or nuclear receptor expression and, thus, provide a more thorough evaluation of the effects of diet on the mechanisms of hypertrophic growth and ventricular dysfunction in the setting of pressure overload. Lastly, whereas the mRNA expression for the fatty acid transporter CD36 did not decrease in the present study, protein expression was not measured. Previous studies observed reduced CD36 protein levels in the spontaneously hypertensive rat,15,16 suggesting that future studies should assess the interaction of diet and hypertension on CD36 protein expression.

Perspectives

The results of the present study demonstrate that a high-fat/low-carbohydrate diet can dramatically attenuate cardiac growth, LV remodeling, contractile dysfunction, and induction of classic molecular markers of cardiac stress that occur in response to hypertension. Because of the high prevalence of hypertension and LVH, the clinical implications of these findings could be profound. There is little information regarding the role of dietary macronutrients in the development of LVH and heart failure in patients with hypertension. Dietary guidelines aimed at prevention of cardiovascular disease
emphasize the importance of consuming a low-fat/high-carbohydrate diet; however, recent findings suggest that reducing fat intake and increasing carbohydrate consumption does not lower the risk of heart disease. On the surface, our results suggest that the optimal diet in hypertension should be low in carbohydrate and high in fat; however, there are no data from humans to support extending this observation into the clinic. In addition, the high sugar content in the standard low-fat rat chow makes it impossible to determine whether the possibility that a low-sugar/high complex carbohydrate/low-fat diet could be just as effective at preventing LVH and contractile dysfunction in hypertension. Future studies must unravel the relationship between dietary fat and carbohydrate intake and the development of LVH and heart failure in patients with hypertension and develop optimal diets for this population.

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Disclosures

None.

References


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