Distinct modulation of angiotensin II-induced early left ventricular hypertrophic gene programming by dietary fat type

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Abstract Long-term dietary fatty acid intake alters the development of left ventricular hypertrophy, but the linking signaling pathways are unclear. We studied the role and underlying signaling mechanisms of dietary fat intake in the early phase of the hypertrophic process. Rats assigned for 4 weeks of high-oil, high-fat, or standard diet were subjected to angiotensin II (Ang II; 33 μg/kg/h, subcutaneous) or vehicle infusion for 24 h. The Ang II-induced increase in left ventricular mRNA levels of hypertrophy-associated genes was higher in rats fed the high-oil diet compared with the standard diet. Western blotting revealed that, in parallel with changes in gene expression, the high-oil diet increased c-Jun N-terminal kinase phosphorylation ($P < 0.001$). Ang II increased p38 mitogen-activated protein kinase (MAPK) phosphorylation in rats fed the high-fat diet (3-fold; $P < 0.01$). The increase in transcription factor activator protein-1 (AP-1) DNA binding activity in response to Ang II was higher in rats fed the high-oil diet compared with those fed the standard diet ($P < 0.001$). Ang II downregulated inducible nitric oxide synthase mRNA levels in fatty acid-supplemented groups compared with the standard diet group. These results show that dietary fat type modulates the early activation of hypertrophic genes in pressure-overloaded myocardium involving the distinct activation of AP-1 and MAPK signal transduction pathways.—Földes, G., S. Vajda, Z. Lakó-Futó, B. Sármán, R. Skoumal, M. Ilves, R. deChâtel, I. Karádi, M. Tóth, H. Ruskoaho, and I. Leprán. **Distinct modulation of angiotensin II-induced early left ventricular hypertrophic gene programming by dietary fat type. *J. Lipid Res.* 2006.** 47: **1219–1226.**

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The prevalence of cardiovascular diseases related to obesity, dyslipidemia, diabetes, and hypertension has reached epidemic levels in industrialized countries. The direct consequences of obesity (i.e., fatty acid overload of cardiac myocytes) may involve cardiomyopathy, arrhythmias, or congestive heart failure (1, 2). Despite the magnitude of the problem, the pathogenesis of myocardial dysfunction in obesity is not well understood. Normal heart obtains at least 60% of its energy from the oxidation of long-chain fatty acids to produce high-energy ATPs (3). The finding that cardiac hypertrophy is associated with a suppression of myocardial fatty acid oxidation and metabolic reversion of the heart toward increased glucose utilization (3, 4) suggests a link between altered myocardial fatty acid metabolism and cardiac hypertrophy. Indeed, in vitro, long-chain fatty acids have been shown to modify angiotensin II (Ang II)-induced hypertrophic responses in cultured neonatal ventricular cardiomyocytes (5). Long-term dietary fatty acid intake also alters the development of left ventricular hypertrophy in vivo (6–10). In addition, pharmacological treatments that decrease cardiac fatty acid utilization have been reported to induce left ventricular hypertrophy in experimental animal models (11). Moreover, many inherited disorders of fatty acid metabolism are accompanied by cardiac hypertrophy and cardiomyopathy (12). However, the signaling processes linking cardiac hypertrophy and hyperlipidemia remain obscure.

In this study, we investigated the role as well as the underlying signaling mechanisms of dietary fat intake in the early phase of the hypertrophic gene program. Thus,

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Abbreviations: Ang II, angiotensin II; ANP, atrial natriuretic peptide; AP-1, activator protein-1; AT_1, angiotensin II type 1 receptor; BNP, B-type natriuretic peptide; BW, body weight; eNOS, endothelial nitric oxide synthase; ERK, extracellular signal-regulated protein kinase; iNOS, inducible nitric oxide synthase; JNK, c-Jun N-terminal protein kinase; LVW, left ventricular weight; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor κB.

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we measured left ventricular mRNA levels of the hypertrophy-associated genes atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), skeletal α-actin, and c-fos in Ang II-induced pressure overload in rats randomly assigned to a standard, high-oil, or high-fat diet. Moreover, the involvement of mitogen-activated protein kinases (MAPKs), c-Jun N-terminal protein kinases (JNKs), extracellular signal-regulated protein kinases (ERKs), and p38 kinase as well as transcription factor activator protein-1 (AP-1) and nuclear factor κB (NF-κB) DNA binding activity in the modulation of the hypertrophic process by dietary fat was characterized by immunoblotting and electrophoretic mobility shift analysis.

MATERIALS AND METHODS

Experimental protocol

Male Sprague-Dawley rats were housed in an experimental animal laboratory with free access to drinking water and rat food pellets. A 6 AM–6 PM on-off environmental light cycle was maintained. Control rats (n = 20) were fed standard laboratory pellets (CRLT/N standard rodent food pellet; fatty acid content, 4.5%; Charles River). Other animals were fed standard pellets supplemented with 10% sunflower seed oil (fatty acid content: saturated palmitic acid, 7%; stearic acid, 5%; monounsaturated oleic acid, 19%; polyunsaturated linoleic acid, 68%; 9 kcal/g) (n = 20) or lard (fatty acid content: saturated palmitic acid, 26%; stearic acid, 14%; myristic acid, 2%; monounsaturated oleic acid, 44%; polyunsaturated linoleic acid, 10%; 9 kcal/g) (n = 20) for 4 weeks. The high-oil diet increases 20:4 and 22:4 (n-6) and decreases 22:5 and 22:6 (n-3) fatty acid content of cardiac ventricular phospholipids, as reported previously (13). The rats were then subjected to Ang II or vehicle infusion for 24 h. Ang II (33 μg/kg/h; Sigma) was administered via osmotic minipumps (Alzet 1003D; pumping rate, 1 μl/h) implanted subcutaneously at the nape of the neck (14). Ang II at this dose markedly increases mean arterial pressure within hours (14). At the end of the infusions, the animals were decapitated, and blood was collected from the abdominal aorta into chilled tubes containing heparin. The plasma was separated by centrifugation at 4°C and kept at −80°C until assayed. Hearts were removed, and the chambers were separated from each other. Left ventricular tissue samples were blotted dry, weighed, immersed in liquid nitrogen, and stored at −80°C until assayed. The experimental design was approved by the Animal Use and Care Committee of Semmelweis University.

Isolation and analysis of mRNA

Total RNA was isolated from left ventricular tissue by the guanidine thiocyanate CsCl method, and 20 μg samples of RNA were transferred to nylon membranes (Osmonics) for Northern blot analysis as described previously (15). Full-length rat ANP cDNA probe, a 390 bp rat BNP cDNA probe, c-fos, and 18S cDNA probe were prepared as reported previously (14). cDNA probes for rat skeletal α-actin and c-fos were made by RT-PCR and ligated to dT-tailed pCR 2.1 vector using the TA Cloning Kit (Invitrogen). Sequencing with the ABI PRISM 310 Genetic Analyzer (Applied Biosystems) confirmed that the probes correspond to bases 2,950–3,184 of rat skeletal α-actin (GenBank accession number V01218) and to bases 231–1,280 of rat c-fos (accession number X06769). cDNA probes were labeled, and the membranes were hybridized and washed as described previously (14).

Real-time quantitative RT-PCR analysis

First-strand cDNA was synthesized from 0.5 μg of total RNA (First Prime Kit; Amersham). Rat endothelial nitric oxide synthase (eNOS), inducible nitric oxide synthase (iNOS), angiotensin II type 1 (AT_1) receptor, and 18S RNA levels were measured by real-time quantitative RT-PCR analysis using Taqman chemistry on an ABI PRISM 7700 Sequence Detection System (Applied Biosystems) as described previously (16). The sequences of the forward (F) and reverse (R) primers and probes (P) for RNA detection were as follows: eNOS (F), 5′-CCTGCCCCCATGACTTTG-3′; eNOS (R), 5′-TCCCGGTAGAGATGGTCCAG-3′; eNOS (P), 5′-FAM-TGTTTGGCTGCCGATGCTCCC-TAMRA-3′; iNOS (F), 5′-GAGGTGGGTGGCCTCGA-3′; iNOS (R), 5′-CCAATCTCGGT-GCCCATG-3′; iNOS (P), 5′-FAM-CCAGCCTGCCCCTTCAATGGT-TG-TAMRA-3′; AT_1 receptor (F), 5′-GTGGCCAAAGTCACCTGCA-3′; AT_1 receptor (R), 5′-GTGGATGACAGCTGGCAAACT-3′; AT_1 receptor (P), 5′-FAM-CATCTGGCTGATGGCTGGCTTGG-TAMRA-3′; 18S (F), 5′-TGGTTGCAAAGCTGAAACTTAAAG-3′; 18S (R), 5′-AGTCAAATTAAGCCGCAGGC-3′; 18S (P), 5′-VIC-CCTGGT-GGTGCCCTTCCGTCA-TAMRA-3′.

Nuclear protein extraction and electrophoretic mobility shift assay

Nuclear extracts were prepared from left ventricular tissue, and protein concentration from each sample was colorimetrically determined (17). Double-stranded synthetic oligonucleotide containing AP-1 or NF-κB motifs of rat BNP promoter was used for analysis of AP-1 or NF-κB DNA binding activity, and a previously described oligonucleotide was used for the measurement of Octamer-1 DNA binding activity (17, 18). Probes were sticky-end-labeled with [α-^{32}P]dCTP by Klenow enzyme. For each reaction mixture (20 μl), 6 μg of nuclear protein and 2 μg of poly(dI-dC) was used in a buffer containing 10 mM HEPES (pH 7.9), 1 mM $MgCl_2$, 50 mM KCl, 1 mM DTT, 1 mM EDTA, 10% glycerol, 0.025% Nonidet P-40, 0.25 mM phenylmethylsulfonyl fluoride, and 1 μg/ml each of leupeptin, pepstatin, and aprotinin. Protein phosphatase inhibitors NaF (50 mM) and Na_3VO_4 (1 mM) were also added to the mixture. Reaction mixtures were incubated with a labeled probe for 20 min, followed by nondenaturating gel electrophoresis on 5% polyacrylamide gels. Subsequently, gels were dried and exposed in a PhosphorImager screen (Molecular Dynamics).

Western blotting

The tissue was homogenized in lysis buffer containing 20 mmol/l Tris (pH 7.5), 150 mmol/l NaCl, 1 mmol/l EDTA, 1 mmol/l EGTA, 1 mmol/l β-glycerophosphate, 2.5 mmol/l sodium pyrophosphate, 1% Triton X-100, 1 mmol/l Na_3VO_4, 2 mmol/l benzamidine, 1 mmol/l phenylmethylsulfonyl fluoride, 50 mmol/l NaF, 1 mmol/l DTT, and 10 μg/ml each of leupeptin, pepstatin, and aprotinin. Western blot analysis was performed using anti-phospho-p38, anti-phospho-p44/42, anti-phospho-JNK, anti-p38, anti-p44/42, and anti-JNK antibodies, as described previously (18). Samples (30 μg) were loaded onto SDS-PAGE gels and transferred to nitrocellulose filters. The membranes were blocked in 5% nonfat milk and incubated with the indicated primary antibody overnight. The same membranes were labeled with nonphospho antibodies after stripping for 30 min at 60°C in stripping buffer (62.5 mmol/l Tris, pH 6.8, 2% SDS, and 100 mmol/l mercaptoethanol). The levels of phospho-p38, total

TABLE 1. Effect of Ang II or vehicle infusion for 24 h on BW and LVW in rats fed the standard, high-oil, or high-fat diet

	Standard Diet		High-Oil Diet		High-Fat Diet	
Variable	Vehicle	Ang II	Vehicle	Ang II	Vehicle	Ang II
BW (g)	429 ± 10	418 ± 7	419 ± 10	417 ± 6	406 ± 6	394 ± 11
LVW (g)	747 ± 20	801 ± 30	732 ± 17	782 ± 21	700 ± 10	727 ± 25
LVW/BW	1.75 ± 0.03	1.9 ± 0.06[a]	1.75 ± 0.04	1.9 ± 0.04[a]	1.72 ± 0.04	1.84 ± 0.03[a]

Ang II, angiotensin II; BW, body weight; LVW, left ventricular weight. Results are means ± SEM (n = 10 in each group).

[a] $P < 0.05$ versus vehicle.

p38, phospho-ERK, total ERK, phospho-JNK, and total JNK were detected by enhanced chemiluminescence.

Measurement of plasma concentrations of lipid fractions

Plasma total cholesterol, triglyceride, and HDL-cholesterol levels were determined by enzymatic methods (Roche).

Statistical analysis

Results are expressed as means ± SEM. The data were analyzed with unpaired Student's *t*-test or one-way ANOVA followed by Tukey's post hoc test, where appropriate. Correlation coefficients were determined using linear regression analysis. Differences at the level of $P < 0.05$ were considered statistically significant.

RESULTS

Body and left ventricular weights, and plasma lipid levels

Body weight (BW), left ventricular weight (LVW), and LVW/BW ratio were similar in all groups after 4 weeks of different diets (**Table 1**). The high-oil and high-fat diets both caused a marked increase in plasma triglyceride levels compared with the standard diet [1.34 ± 0.17 (n = 8) and 1.50 ± 0.24 (n = 6) mmol/l vs. 0.59 ± 0.07 mmol/l (n = 5); both $P < 0.01$]. Total cholesterol levels decreased after the high-oil diet compared with the high-fat or standard diet (1.25 ± 0.07 vs. 1.5 ± 0.12 and 1.58 ± 0.12 mmol/l; both $P < 0.05$), whereas plasma HDL-cholesterol levels were similar in all groups [1.10 ± 0.08 mmol/l (n = 6 in each group)]. Atherogenic index (the ratio of total cholesterol to HDL-cholesterol) was also significantly lower in the high-oil diet group than in the high-fat and standard diet groups (1.26 ± 0.02 vs. 1.45 ± 0.08 and 1.38 ± 0.05; both $P < 0.05$).

Left ventricular hypertrophy and gene expression in response to Ang II infusion

A hypothetical model for the integration of dietary fat and Ang II signaling pathways is presented in **Fig. 1**. Ang II infusion similarly increased LVW/BW ratios in rats maintained on the standard, high-oil, or high-fat diet ($P < 0.05$ vs. vehicle-infused rats) (Table 1). Left ventricular c-fos mRNA levels were increased 4-fold in rats fed the standard diet in response to 24 h of Ang II infusion (**Fig. 2A, B**). The increase in c-fos mRNA levels was higher in rats fed the high-fat diet than in those fed the high-oil and standard diets ($P < 0.05$) (Fig. 2B). Ang II infusion also resulted in a 3-fold increase in left ventricular ANP mRNA levels in rats fed the standard diet, and there was a tendency for BNP mRNA levels to increase (1.5-fold); however, this change was not statistically significant ($P = 0.1$) in rats fed the standard diet (Fig. 2A, C, D). Ang II produced a higher induction of ANP expression in rats fed the high-oil than the high-fat diet ($P < 0.01$) (Fig. 2C). The increase in left ventricular BNP mRNA levels in response to Ang II infusion was markedly higher in rats fed the high-oil diet compared with those fed the high-fat or standard diet ($P < 0.001$) (Fig. 2D). Left ventricular skeletal α-actin mRNA level was increased 4-fold in response to Ang II infusion in rats fed the standard diet and was significantly higher in rats fed the high-oil diet compared with those fed the high-fat diet ($P < 0.05$) (Fig. 2A, E). Thus, the Ang II-induced increases in left ventricular ANP, BNP, and skeletal α-actin mRNA levels were highest in rats fed the high-oil diet, whereas c-fos mRNA levels were increased mostly in rats fed the high-fat diet (Fig. 2).

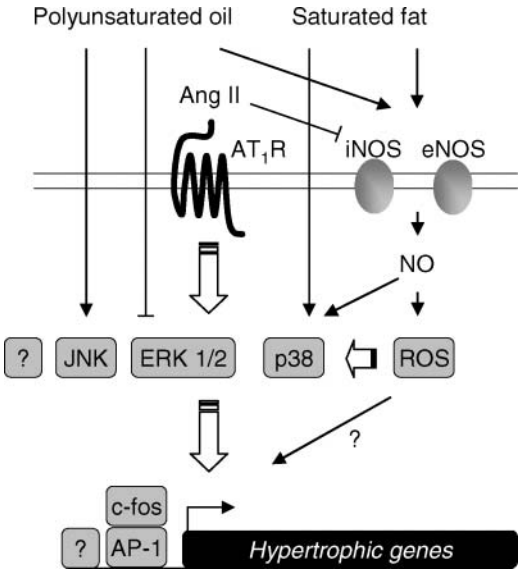

Fig. 1. Hypothetical model for the integration of dietary fat and angiotensin II (Ang II) signaling via mitogen-activated protein kinases (MAPKs) and transcription factor activator protein-1 (AP-1) pathways. eNOS, endothelial nitric oxide synthase; ERK, extracellular signal-regulated protein kinase; iNOS, inducible nitric oxide synthase; JNK, c-Jun N-terminal protein kinase; NO, nitric oxide; ROS, reactive oxygen species.

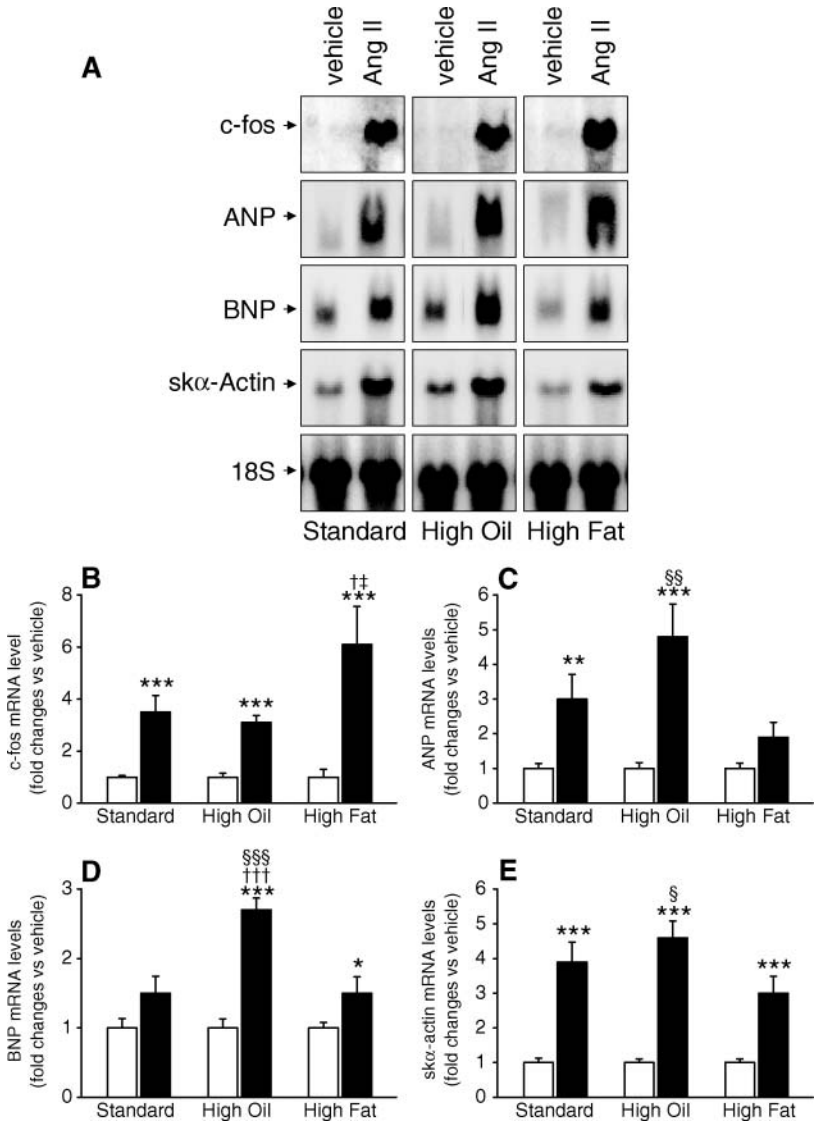

Fig. 2. A: Northern blot analysis showing the effect of Ang II or vehicle infusion for 24 h on left ventricular c-fos, atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), and skeletal (sk) α-actin mRNA levels in rats fed the standard, high-oil, or high-fat diet. Twenty micrograms of RNA was loaded onto each lane, and the blot was sequentially hybridized with ^{32}P-labeled cDNA probes. Hybridization signals for ribosomal 18S RNA are also shown. B–E: Bar graphs show left ventricular c-fos (B), ANP (C), BNP (D), and skeletal α-actin (E) mRNA levels in response to vehicle (open bars) or Ang II (closed bars) infusion in rats fed the standard, high-oil, or high-fat diet. mRNA results are expressed as the ratio of mRNA to 18S. Results are means ± SEM (n = 10). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus vehicle; † $P < 0.05$, ††† $P < 0.001$ versus the standard diet; ‡ $P < 0.05$ versus the high-oil diet; § $P < 0.05$, §§ $P < 0.01$, §§§ $P < 0.001$ versus the high-fat diet.

Left ventricular iNOS, eNOS, and AT_1 receptor mRNA levels

To investigate the possible signaling mechanisms underlying the modulation of hypertrophic gene programming by dietary fat type, we measured mRNA levels of iNOS, a major factor in both fatty acid metabolism and cardiac hypertrophy (19, 20). As assessed by RT-PCR analysis, baseline left ventricular iNOS mRNA levels were significantly higher in rats fed the high-oil (2.7-fold; $P < 0.05$) or the high-fat (4.7-fold; $P < 0.01$) diet than in those fed the standard diet (**Fig. 3A**). Also, baseline eNOS mRNA levels were higher in the high-fat diet group than in the standard diet group (1.6-fold; $P < 0.01$) (Fig. 3B). Baseline eNOS mRNA levels correlated with c-fos mRNA levels ($R = 0.6$, n = 17, $P < 0.01$). Ang II infusion for 24 h markedly decreased iNOS mRNA levels in the left ventricle in the high-oil and high-fat diet groups, whereas eNOS mRNA levels remained unchanged in all three groups. The decrease in iNOS mRNA levels in the left ventricle correlated inversely with LVW/BW ratio ($R = 0.5$, n = 37, $P < 0.01$) and was most prominent in the high-fat diet group (Fig. 3A). There was a tendency for AT_1 receptor mRNA levels to decrease in response to Ang II in all three groups, although these changes in AT_1 receptor expression were not statistically significant (Fig. 3C). Together, high-oil intake increased iNOS mRNA levels, and high-fat intake increased both iNOS and eNOS gene expression in the left ventricle. Ang II infusion downregulated iNOS mRNA levels in fatty acid-supplemented groups (Fig. 1).

MAPKs in the left ventricle

To study the involvement of MAPKs in the dietary modulation of hypertrophic responses to Ang II infusion, Western blotting using phospho-p38-, phospho-p44/p42-, and phospho-JNK-specific antibodies was performed. Baseline left ventricular JNK activity was 1.6-fold higher ($P < 0.01$) and ERK 1/2 activity was 3-fold lower ($P < 0.05$) in rats fed the high-oil diet compared with those fed the standard or high-fat diet (**Fig. 4A–C**), whereas baseline p38 MAPK activity did not differ between groups (Fig. 4A, D).

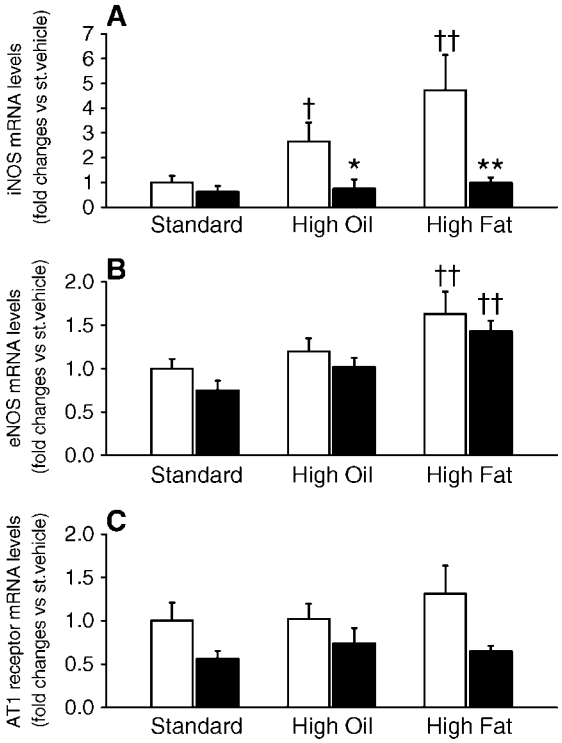

Fig. 3. Effect of 24 h vehicle (open bars) or Ang II (closed bars) infusion on left ventricular iNOS (A), eNOS (B), and Ang II receptor type 1 (AT1; C) mRNA levels in rats fed the standard, high-oil, or high-fat diet. mRNA results are expressed as the ratio of mRNA to 18S. Results are shown as fold changes versus vehicle-infused rats fed the standard diet and are means ± SEM (n = 10). * $P < 0.05$, ** $P < 0.01$ versus vehicle; † $P < 0.05$, †† $P < 0.01$ versus the standard diet.

Ang II infusion for 24 h had no effect on ERK 1/2 phosphorylation, whereas JNK activity decreased significantly in rats fed the standard diet in response to Ang II infusion ($P < 0.01$). Moreover, Ang II infusion produced a 3-fold increase in p38 MAPK phosphorylation in rats fed the high-fat diet ($P < 0.01$) but not in rats fed the high-oil or standard diet (Fig. 4A, D). A significant correlation was observed between changes in p38 MAPK activity and c-fos mRNA ($R = 0.6$, n = 16, $P < 0.01$) and iNOS mRNA ($R = -0.8$, n = 7, $P < 0.05$) levels. Collectively, baseline left ventricular JNK activity was increased and ERK 1/2 activity was suppressed with the high-oil diet. Ang II markedly increased p38 MAPK activity in rats fed the high-fat diet (Fig. 1).

Left ventricular AP-1 binding activity

As assessed by electrophoretic mobility shift assay, baseline DNA binding activity of nuclear extracts and double-stranded oligonucleotide probe containing the BNP AP-1 sites was similar in rats fed the high-oil diet and the high-fat diet compared with the standard diet (**Fig. 5A, B**). Ang II infusion resulted in 1.7-, 2.3-, and 2.5-fold increases in AP-1 DNA binding activity sites in rats fed the standard, high-oil, and high-fat diets, respectively. The increase in left ventricular AP-1 DNA binding activity in response to Ang II infusion was higher in rats fed the high-

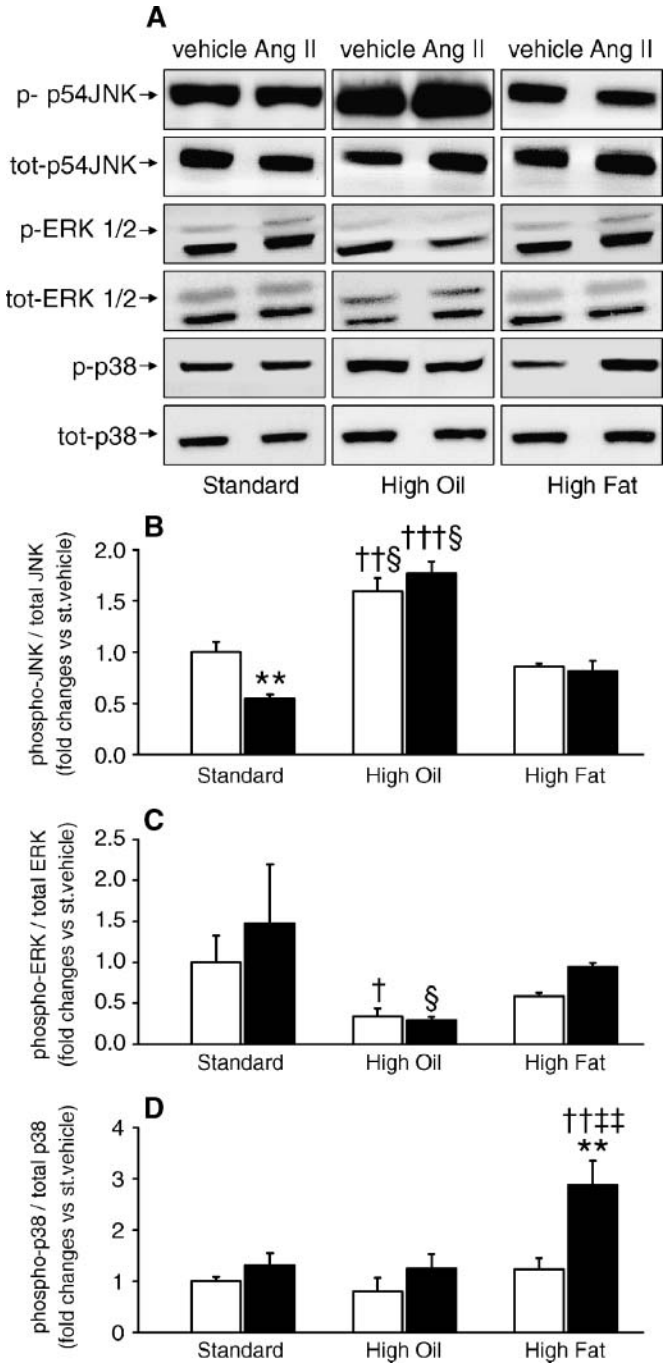

Fig. 4. A: Western blot analysis showing the effect of 24 h vehicle or Ang II infusion on left ventricular activity of JNK, ERK 1/2, and p38 in rats fed the standard, high-oil, or high-fat diet. The cytoplasmic protein was extracted, and 30 μg samples of protein were boiled, resolved by SDS-PAGE, and blotted onto nitrocellulose membranes. The blots were incubated with phospho-JNK-, phospho-p44/p42-, phospho-p38-, JNK-, p44/42-, and p38-specific antibodies and detected by enhanced chemiluminescence. B–D: Bar graphs show the effect of 24 h vehicle (open bars) and Ang II (closed bars) infusion on left ventricular activity of JNK (B), ERK 1/2 (C), and p38 (D) in rats fed the standard, high-oil, or high-fat diet. Results are expressed as the ratio of the phosphorylated protein kinase and total protein kinase, as assessed by Western blot analysis. Results are shown as fold changes versus vehicle-infused rats fed the standard diet and are means ± SEM (n = 3–5). ** $P < 0.01$ versus vehicle; † $P < 0.05$, †† $P < 0.01$, ††† $P < 0.01$ versus the standard diet; ‡‡ $P < 0.01$ versus the high-oil diet; § $P < 0.001$ versus the high-fat diet.

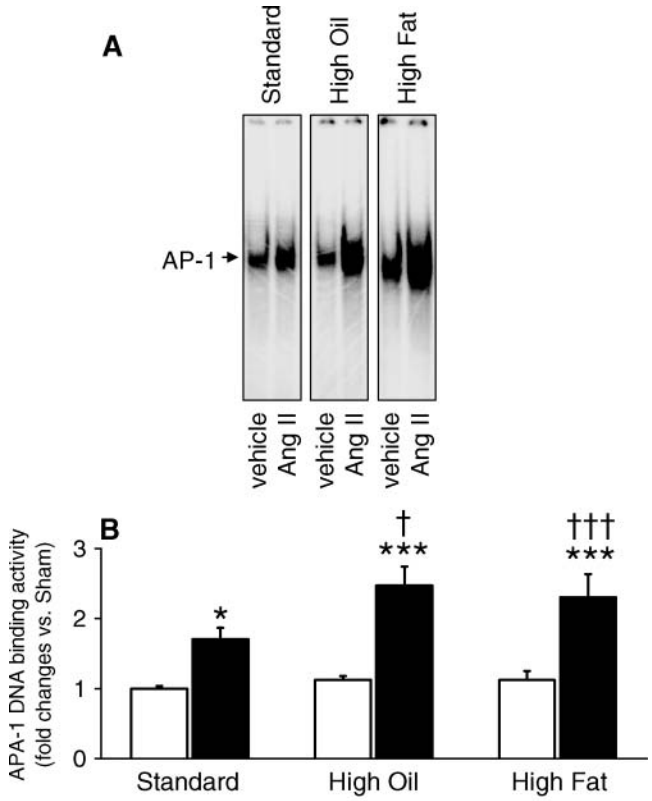

Fig. 5. A: Gel mobility shift assays showing the effect of 24 h vehicle and Ang II infusion on BNP AP-1 DNA binding in rats fed the standard, high-oil, or high-fat diet. Nuclear protein extraction and gel mobility shift assays were performed as described in Materials and Methods. The nuclear extracts from left ventricular tissue were incubated with rBNP-90 oligonucleotide probe. B: Effect of 24 h vehicle (open bars) and Ang II (closed bars) infusion on BNP AP-1 binding activity. Results are means ± SEM (n = 3–5). * $P < 0.05$, *** $P < 0.001$ versus vehicle; † $P < 0.05$, ††† $P < 0.01$ versus the standard diet.

oil diet compared with those fed the standard diet ($P < 0.001$) (Fig. 5B). The Ang II-induced increase in AP-1 DNA binding activity correlated significantly with changes in the mRNA levels of c-fos ($R = 0.7$, n = 38, $P < 0.001$), BNP ($R = 0.5$, n = 38, $P < 0.001$), and skeletal α-actin ($R = 0.5$, n = 39, $P < 0.001$). Thus, fatty acid intake resulted in a further increase in AP-1 binding activity in response to Ang II (Fig. 1). The DNA binding activity of NF-κB transcription factor did not alter in response to 24 h Ang II infusion (data not shown).

DISCUSSION

Given that fatty acids represent the main source of energy of hearts and that decreased fatty acid utilization has been suggested to play a role in the pathophysiology of cardiac hypertrophy (3), we hypothesized that dietary supplementation of fatty acids may influence the early hypertrophic process and activation of cardiac gene expression produced by pressure overload. The results presented here show that excess feeding of saturated or polyunsaturated fatty acids distinctly modified the expression of the hypertrophy-associated genes ANP, BNP, and skeletal α-actin in response to Ang II-induced pressure overload. A major finding of the study was that Ang II in rats fed with increased polyunsaturated n-6 fatty acid (i.e., linoleic acid) induced a more pronounced increase in ANP, BNP, and skeletal α-actin genes compared with that in rats fed the saturated fat diet, whereas the latter diet did not significantly modify Ang II-induced hypertrophic gene programming. It has been reported that increased saturation of dietary fat results in increases of systolic and diastolic blood pressure in humans and rats (21, 22). On the other hand, diets with polyunsaturated n-6 fatty acids exert an antihypertensive effect on Ang II-induced hypertension in rats (23). Thus, the modulatory effect of the high-oil diet on the hypertrophic gene program may be independent of alterations in pressure overload. The distinct modulation of ANP, BNP, and skeletal α-actin gene expression by dietary fat type also cannot be explained by alterations in AT_1 receptor expression, because AT_1 receptor mRNA levels were similar with fatty acid and standard diets. Other membrane-associated molecules, such as small G proteins of the Rho family, may also be involved in the hypertrophic process by activating downstream signaling molecules, including MAPKs. In support of this, the cholesterol-lowering statins have been reported to inhibit hypertrophy via RhoA-dependent pathways (24). The Ang II-induced increase in left ventricular levels of c-fos was higher in rats fed the high-fat diet than in those fed the standard diet, whereas polyunsaturated fatty acid alone did not change c-fos expression in the left ventricle, in agreement with previous findings (25).

One potential mechanism by which altered lipid metabolism may lead to ventricular hypertrophy and dysfunction in rats is increased myocardial oxidative stress mediated by nitric oxide synthase (26). In this study, high oil intake increased baseline iNOS mRNA levels and high fat intake increased both iNOS and eNOS expression. High myocardial expression of iNOS and eNOS, and the resulting increase in nitric oxide production, has been suggested to mediate the cardiotoxic effects of high-fat and high-oil diets via increased formation of intracellular toxins, peroxisomal-generated H_2O_2, or other reactive oxygen species (26). Indeed, higher baseline levels of eNOS was closely associated with increased c-fos levels. Of note, a previous report has shown that despite the beneficial effects on serum lipids, a high-oil diet leads to markedly higher levels of oxidative stress than a saturated fat diet in rats (27). Moreover, we observed that Ang II infusion markedly downregulated iNOS mRNA levels in fatty acid-supplemented groups compared with the standard diet group. As nitric oxide attenuates ventricular natriuretic peptide expression (28), Ang II-induced inhibition of nitric oxide synthesis may contribute to the upregulation of ANP and BNP gene expression in the rat heart.

Ang II have been shown to activate the AP-1 nuclear transcription complex (29). AP-1 regulates the expression of various genes by binding AP-1 consensus sequences present in their promoter regions, leading to coordinated

increases in gene expression. Here, we show that AP-1 activation is associated with increased c-fos, ANP, BNP, and skeletal α-actin gene expression in response to Ang II infusion. Interestingly, excess fatty acid intake resulted in a further increase in AP-1 binding activity in response to Ang II, suggesting that AP-1 may play a role in dietary fat-induced transcription factor regulation. In addition to AP-1, the fatty acid-inducible transcription factors NF-κB and peroxisome proliferator-activated receptors have been demonstrated to be involved in the hypertrophic response of cultured neonatal rat cardiomyocytes and in a chronic pressure overload model in vivo (30, 31). In this study, NF-κB activity was unchanged at 24 h of Ang II infusion. Whether alternative pathways that may lead to ANP and BNP induction, such as calcineurin/NFAT, JAK/STAT, or GATA factors (18, 32), are involved in the control of gene programming by dietary fat type remains to be determined.

The intracellular signaling cascades that link the hypertrophic stimuli and dietary fatty acid changes into the activation of transcription factors and cardiac gene expression are poorly understood. At present, only the protein kinase C pathway has been shown to be activated by dietary fat and to accompany cardiac hypertrophy in rats (33). MAPKs are a family of protein kinases that regulate a wide array of cellular processes in response to extracellular stimuli, including cell growth and apoptosis (32, 34). Previous studies have demonstrated that signal transduction through JNK, ERK 1/2, and p38 MAPKs modulates cardiac hypertrophy in pressure-overloaded rat myocardium (35, 36). Because MAPKs may be involved in fatty acid homeostasis (37), we tested the hypothesis that the type of dietary fat may modulate cardiac MAPK activation and thereby the hypertrophic process in the rat myocardium. Indeed, here, we observed that in parallel with increased ANP and BNP gene expression, baseline left ventricular JNK activity was increased significantly only with the high-oil diet, suggesting that the JNK pathway may play a role in mediating the effects of dietary polyunsaturated fats on the hypertrophic process. Moreover, JNK activity did not decrease in the high-oil diet group in response to Ang II infusion, as occurred in the standard diet group. Thus, our results suggest that the transcriptional activation of hypertrophic genes such as ANP and BNP in response to the high-oil diet may be mediated via increased JNK activity. In contrast to JNK, ERK 1/2 activity was suppressed by the high-oil diet. Ang II, which has been shown to activate ERK 1/2 transiently within hours in conscious rats (29, 38), did not increase its activity at 24 h.

In contrast to JNK and ERK 1/2, the third branch of MAPKs, p38 MAPK activity, was similar in all dietary groups, and Ang II markedly increased p38 MAPK activity only in rats fed the high-fat diet. Active p38 MAPK may have several detrimental vascular effects and is known to induce pathways that promote cellular lipid uptake (37). Among the multiple signaling pathways activated by Ang II, enhanced activation of p38 MAPK (39) might be a component mediating the inhibitory effect of Ang II on fatty acid induction of iNOS expression. In agreement with this, previous studies have also provided evidence that p38 kinase pathways are necessary for regulating iNOS expression in the myocardial tissue (40, 41). The highest induction of p38 and the associated downregulation of iNOS, however, only weakly correlated with left ventricular hypertrophy or ANP mRNA levels in rats fed the high-fat diet. Thus, our results suggest that p38 MAPK and iNOS may mediate primarily dietary fat- and Ang II-induced processes independent of hypertrophy itself. Furthermore, a significantly higher increase in c-fos mRNA levels in the left ventricle in the high-fat group in response to Ang II infusion may be mediated by activation of the p38 kinase and AP-1 pathways, because increased mRNA levels of c-fos correlated with p38 kinase and AP-1 DNA binding activity.

We have shown that dietary fat type modulates the early activation of hypertrophic genes in pressure-overloaded myocardium and involves the distinct activation of AP-1 and MAPK signal transduction pathways (Fig. 1). Our observations suggest a previously unrecognized integration of signaling by Ang II and dietary fatty acids. Because the intake of saturated fats and plant seed n-6 polyunsaturated fatty acids has increased in Western-type diets, our results could have clinically relevant implications for humans. If similar mechanisms are observed in clustered human counterparts of diet-induced obesity and hypertension, attenuation or reversal of the progression of associated cardiac hypertrophy is likely to require an integrated approach, with alteration of the dietary fat profile being a logical target. The exact signaling mechanisms involved in the control of energy metabolism in the normal and diseased heart remain to be determined. It will also be of interest to explore whether dietary fat type influences cardiac hypertrophy in the long term and in other experimental models of pressure overload, such as aortic banding.

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