Original Article

Oleic Acid Attenuates Ang II (Angiotensin II)-Induced Cardiac Remodeling by Inhibiting FGF23 (Fibroblast Growth Factor 23) Expression in Mice

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Abstract—Plasma metabolic profiles were compared between patients with hypertension with and without left ventricular hypertrophy and significantly decreased oleic acid (OA) levels were observed in the peripheral blood of patients with hypertension with left ventricular hypertrophy. We sought to determine the effect and underlying mechanisms of OA on cardiac remodeling. In vitro studies with isolated neonatal mouse cardiomyocytes and cardiac fibroblasts revealed that OA significantly attenuated Ang II (angiotensin II)-induced cardiomyocyte growth and cardiac fibroblast collagen expression. In vivo, cardiac function, hypertrophic growth of cardiomyocytes, and fibrosis were analyzed after an Ang II (1000 ng/kg/minute) pump was implanted for 14 days. We found that OA could significantly prevent Ang II-induced cardiac remodeling in mice. RNA sequencing served as a gene expression roadmap highlighting gene expression changes in the hearts of Ang II-induced mice and OA-treated mice. The results revealed that FGF23 (fibroblast growth factor 23) expression was significantly upregulated in mouse hearts in response to Ang II infusion, which was significantly suppressed in the hearts of OA-treated mice. Furthermore, overexpression of FGF23 in the heart by injection of an AAV-9 vector aggravated Ang II-induced cardiac remodeling and impaired the protective effect of OA on cardiac remodeling. Further study found that OA could suppress Ang II-induced FGF23 expression by inhibiting the translocation of Nurr1 (nuclear receptor–related 1 protein) from the cytoplasm to the nucleus. Our findings suggest a novel role of OA in preventing Ang II-induced cardiac remodeling via suppression of FGF23 expression. **(*Hypertension*. 2020;75:00-00. DOI: 10.1161/HYPERTENSIONAHA.119.14167.)** ● **Online Data Supplement**

Key Words: cytoplasm ■ hypertension ■ oleic acid ■ prevalence ■ transcriptome

Hypertension can lead to damage of various target organs, while the prevalence of left ventricular hypertrophy (LVH) in mild-to-moderate hypertensive populations is estimated to be 20% to 50% in various studies.[1–3] However, the pathological mechanism of hypertensive cardiac remodeling remains poorly understood. Previous studies indicated that circulating molecules play a critical role in the pathological process of hypertensive cardiac remodeling.[4] On one hand, circulating metabolites acting as ligands could activate signaling pathways related to cardiac remodeling.[5,6] On the contrary, a specific pattern of plasma metabolites that reflected changes in pathophysiologic mechanisms of hypertensive cardiac remodeling was reported in many studies.[7,8] Therefore, we detected the plasma metabolic profiles in patients with hypertension with and without LVH to evaluate the metabolic characteristics of patients with hypertension with LVH and discover critical metabolites that regulate the pathological processes of cardiac remodeling.

Metabolomics is a novel technology for discovering intermediate and end metabolites of physiological processes and is a powerful tool for uncovering metabolic pathways and biomarkers associated with cardiovascular disease.[9] Dynamic changes in hundreds of low-molecular weight metabolites in peripheral blood have been identified as the main characteristic for distinguishing physiological and disease states, such as diabetes mellitus[10] and systemic cardiovascular disorders,[11] and predicting clinical outcomes.[12] Fan et al[13] used a nontargeted metabolomics method and found that differences in small-molecule metabolites in peripheral blood may reflect underlying coronary artery disease and serve as biomarkers for coronary artery disease progression. Unlike other organs, changes in the metabolic mode of the myocardium are closely associated with pathological and physiological cardiac remodeling.[14,15] Few metabolomics studies have been performed in patients with cardiac hypertrophy or heart failure to screen biomarkers and therapeutic targets. Moreover, the detailed

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mechanisms by which differential metabolites derived from animal models influence the pathological processes of cardiovascular disease remain unclear. Therefore, analyzing metabolic profile characteristics can reveal systemic metabolic alterations and significantly differential metabolites in patients with cardiac hypertrophy, which can be used to identify novel biomarkers and potential therapeutic targets.

OA plays a critical role as an important monounsaturated fatty acid in regulating fatty acid intake and oxidative phosphorylation.[16,17] Many randomized clinical trials have reported that an OA-rich Mediterranean diet decreases the incidence of breast cancer, age-related cognitive decline, and type 2 diabetes mellitus[18,19] and reduces the risk of cardiovascular events, including coronary heart disease, stroke, heart failure, and myocardial infarction.[20] Furthermore, OA alone can regulate atrial electrophysiological characteristics in the context of calcium and sodium dysregulation and induce the release of glucagon-like peptide-1.[21,22] However, the role and mechanisms of OA in hypertensive cardiac remodeling remain unclear.

In the present study, we sought to test the hypothesis that OA plays a protective role in cardiac remodeling. To examine this, we observed cardiac remodeling and function in an Ang II (angiotensin II)-infused mouse model. OA prevented pathological cardiac remodeling in mice by inhibiting FGF23 (fibroblast growth factor 23) expression in the heart.

Materials and Methods

This article adheres to the American Heart Association Journals implementation of the Transparency and Openness Promotion Guidelines. The data that support the findings of this study are available from the corresponding author on reasonable request. Detailed methods are available in the online-only Data Supplement.

Patients

The study protocol was reviewed and approved by the Human Ethics Committee, Fuwai Hospital (Approval No.: 2016-732), and the study was conducted in accordance with the principles of Good Clinical Practice and the Declaration of Helsinki. All participants gave written informed consent.

We enrolled 54 patients with hypertension without LVH and 38 patients with hypertension with LVH from the sample library produced under national basic research grant of China G2000056901. Echocardiography was used to measure the structural parameters of the patients' hearts, including left ventricular internal dimension at end diastole and end systole (LVIDD and LVISD), interventricular septal thickness (IVST), and posterior wall thicknesses (PWTs). LVM (left ventricular mass) was calculated according to the following formula: LVM (g)=0.8×[1.04×(LVIDD+IVST+PWT)3-(LVID-(LVIDD)3]+0.6 LVM was indexed to BSA (body surface area) to obtain LVM body surface area. LVH was defined as previously described.[23] LVH was defined using the partition values of LVM body surface area above 125 g/m^2 for men and above 120 g/m^2 for women. Severe systemic diseases (collagenosis, endocrine and metabolic diseases [except for diabetes mellitus], inflammation, and liver, neoplastic, or renal diseases) were criteria for exclusion. Medical histories, including prescriptions for medication use, blood biochemical indicators, and lifestyle habits (smoking and drinking), were recorded carefully, and the baseline characteristics of the patients with hypertension without LVH and patients with hypertension with LVH are shown in the Table S1 in the online-only Data Supplement. Sample preparation and LC-Q/TOF-MS-based nontargeted metabolomics were performed according to a previous report.[13]

Other targeted metabolomics were performed to validate nontargeted metabolomics results in a second cohort as a validation set, including 50 patients with hypertension with LVH and 50 patients with hypertension without LVH. The baseline characteristics of the validation set are shown in the Table S2.

Animals

Animal experiments were approved by the Committee of Fuwai Hospital on Ethics of Animal Experiments (Approval No.: 0085-M-200-HX) and complied with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals, and the manuscript adheres to the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines for reporting animal experiments. Male C57BL/6 mice (10 weeks) were purchased from the Model Animal Research Center of Nanjing University (Nanjing, China). All mice were housed in a specific-pathogen-free environment under a 12 hour/12 hour light-dark cycle and fed rodent diet ad libitum. No specific statistical tests were performed to predetermine the sample size. Sample size was chosen according to relevant references and experiences. Animals were randomly assigned to experimental groups by using the random number generator function in SPSS.[24] Investigators blinded to the treatment groups evaluated outcomes in all mice and performed the analysis. For mouse euthanasia, 3.0% isoflurane mixed with oxygen (100%, airflow velocity: 1 L/minute) was used as inhalable anesthesia in mouse and then anesthetized animals were sacrificed by cervical dislocation. Heart was rapidly excised and prepared for histology, proteins, and RNA extraction.

Animal Model of Cardiac Remodeling

After mice orally received OA (160 mg/kg; body weight, 0.3 mL/mouse) or saline for 14 days, cardiac remodeling was induced by chronic infusion of Ang II (Sigma-Aldrich) at a dose of 1000 ng/kg per minute for 2 weeks with Alzet 2004 pumps.[25] In brief, according to the manufacturer's protocol, the prefilled pump was incubated in sterile saline at 37°C for 48 hours. Mice were anesthetized with 3.0% isoflurane mixed with oxygen (100%, airflow velocity: 1 L/minute) was used to maintain anesthesia. An incision was made in the skin on the back of the mouse, and the pump was implanted into the subcutaneous tissue. After being sutured and regaining consciousness, the mice were returned to their original cages and fed individually until the end of the experiment.

Statistical Analysis

The mean±SD was calculated for all of the data. Statistical comparisons for percentages were performed using χ^2 analysis. Statistical differences were calculated with the 2-tailed Student t test when comparing 2 conditions, and ANOVA was used when comparing >2 conditions. For parametric data with equal variance, 1-way ANOVA with Tukey post hoc test was used. For parametric data with unequal variance, 1-way ANOVA with the Games-Howell post hoc test was used. A P value <0.05 was considered statistically significant. All statistical analyses were carried out using GraphPad Prism software (Version 5.0, GraphPad Software, Inc, San Diego, CA) and IBM SPSS Statistics 21.0 (IBM Corporation, Armonk, NY).

Results

OA Levels Were Significantly Decreased in the Peripheral Blood of Patients with Hypertension With LVH compared With Patients with Hypertension Without LVH

To determine whether the serum metabolic profiles of patients with hypertension with LVH were different from those of patients with hypertension without LVH, LC-Q/TOF-MS-based nontargeted metabolomics was performed. A total of 741 and 972 features were profiled in positive and negative modes, respectively (n=92). Then, multivariate statistical analysis using the principal component analysis model was performed on the LC-Q/TOF-MS spectra results of the

plasma samples. The principal component analysis score plots revealed clear discrimination for patients with hypertension with LVH versus patients with hypertension without LVH and without overfitting (Figure 1A; Figure S1). The following criteria were satisfied to screen differential metabolites between patients: (1) significant differences in the 2 comparisons, P<0.05 and a false discovery rate <0.05; (2) variable importance in the projection values >1. Ultimately, a total of 14 metabolites were found in positive and negative modes (Figure 1B; Table S4). Binary logistic regression analysis and an optimized algorithm of the forward stepwise (Wald) method were used to construct the best model using these 14 metabolites.26,27 Seven metabolites had close associations with hypertensive LVH, including eicosapentaenoic acid, OA, HOME (hydroxy-octadecenoic acid), linoleic acid, PC (22:6; phosphatidylcholine 22:6), citric acid, and PC (20:3).

Metabolite importance was detected by multiple runs of a random forest.28 The random forest importance measures mean decrease accuracy and mean decrease Gini were calculated in 400 trees, and the results showed that eicosapentaenoic acid and OA ranked in front of the others (Figure S2). A previous study showed a clear protective effect of

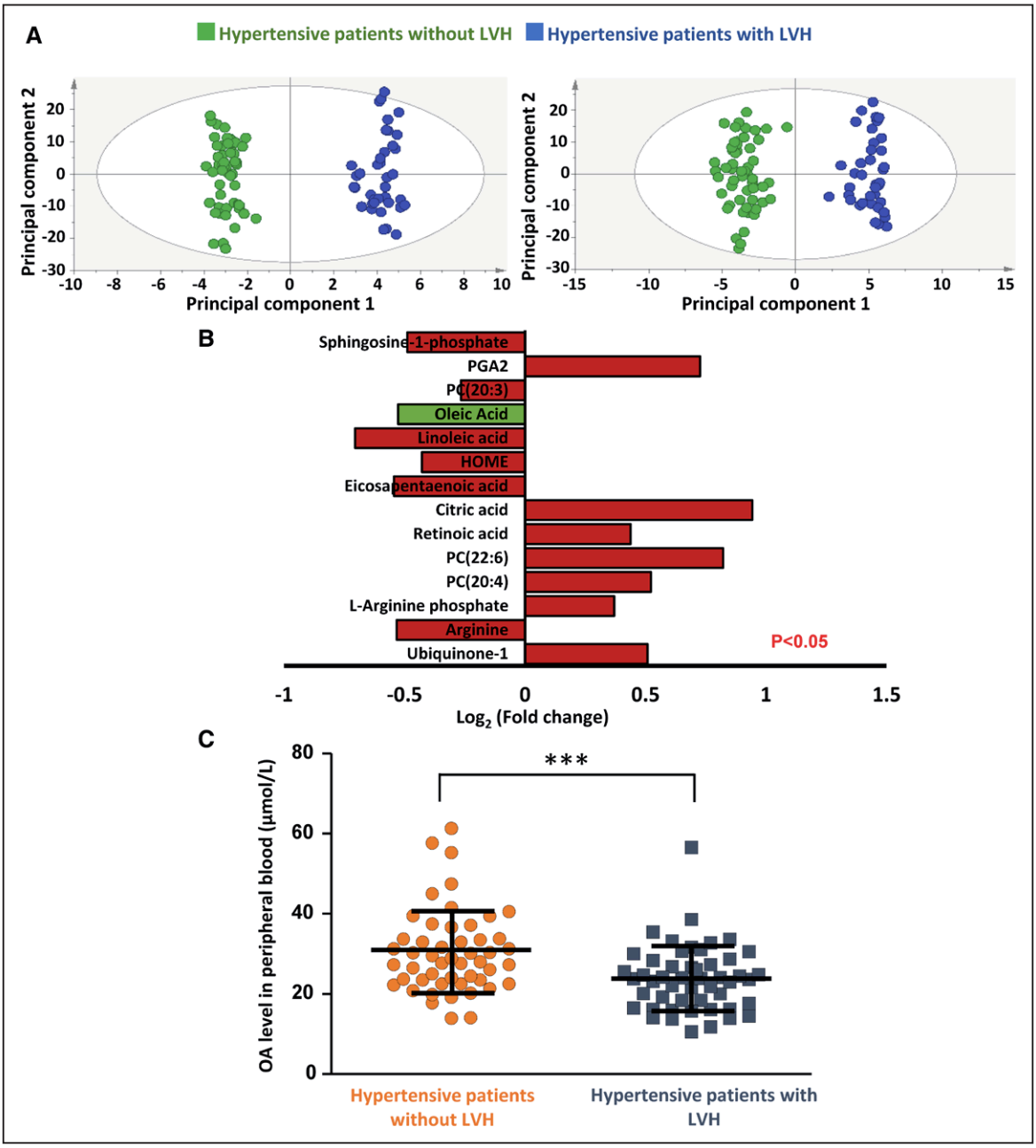

Figure 1. Oleic acid (OA) levels were significantly decreased in the peripheral blood of patients with hypertension with left ventricular hypertrophy (LVH) compared with patients with hypertension without LVH. **A**, The orthogonal projection to latent structures-discriminant analysis score plots between patients with hypertension with and without cardiac hypertrophy (left: positive mode, right: negative mode). **B**, Fourteen metabolites with significant differences between patients with hypertension with and without LVH were found by principal component analysis and Student *t* test. Fold change is greater, levels of that metabolite are higher in patients with hypertension with LVH. **C**, OA levels in the peripheral blood of patients with hypertension with and without LVH in the validation set, data are presented as the mean±SD, n=50, unpaired Student (2-tailed) *t* test, ***P<0.001.

eicosapentaenoic acid on multiple causes–induced cardiac remodeling, further validating the accuracy of our analytical method.[29,30] OA, as an important monounsaturated fatty acid, plays a key role in regulating energy metabolism, especially for fatty acid oxidation. However, the effect of OA on Ang II-induced cardiac remodeling remains unclear. We therefore decided to validate plasma OA levels in peripheral blood in the validation set by targeted metabolomics technology. Indeed, a significantly decreased OA level was found in the peripheral blood of patients with hypertension with LVH compared with those without LVH (Figure 1C). In addition, many large-scale, multicenter clinical studies have suggested that an OA-rich mediterranean diet is associated with better cardiovascular health outcomes.[31,32] Therefore, we decided to study the effect of OA on hypertensive cardiac remodeling and to evaluate the possible mechanism in vitro and in vivo.

OA Attenuated Ang II-Induced Cardiomyocyte Growth and CF Collagen Expression In Vitro

We first examined the effect of OA on Ang II-induced cardiomyocyte growth and CF collagen expression in vitro. Immunostaining of neonatal mouse cardiomyocytes for cytoskeleton with fluorescently labeled phalloidin indicated that OA significantly inhibited the hypertrophic growth of cardiomyocytes in response to Ang II (Figure 2A). In addition, OA was able to significantly inhibit ANP (atrial natriuretic peptide) and BNP (brain natriuretic peptide) mRNA levels in cardiomyocytes following 24 hours of Ang II administration (Figure 2B). In cardiac fibroblasts (CFs), the increased expression of the fibrotic markers α-SMA (α-smooth muscle actin), Col1a1, and Col3a1 following Ang II administration was prevented by OA treatment (Figure 2C). These data demonstrated that OA prevented Ang II-induced cardiomyocyte growth and CF collagen expression in vitro.

OA Ameliorated Ang II-Induced Cardiac Remodeling In Vivo

To determine whether in vivo OA can inhibit Ang II-induced cardiac remodeling, we used mice to observe cardiac remodeling and OA function in an Ang II-infused model. Ang II-induced mice had significantly higher systolic blood pressure than vehicle-treated mice after 2 weeks of Ang II infusion. There were no differences between Ang II-induced mice and OA-treated mice in systolic blood pressure during the experimental period (Figure S3). Echocardiography identified a decreased ejection fraction (EF%) and increased E/E' in the hearts of Ang II-induced mice, while OA treatment attenuated these alterations (Figure 3A and 3B; Table

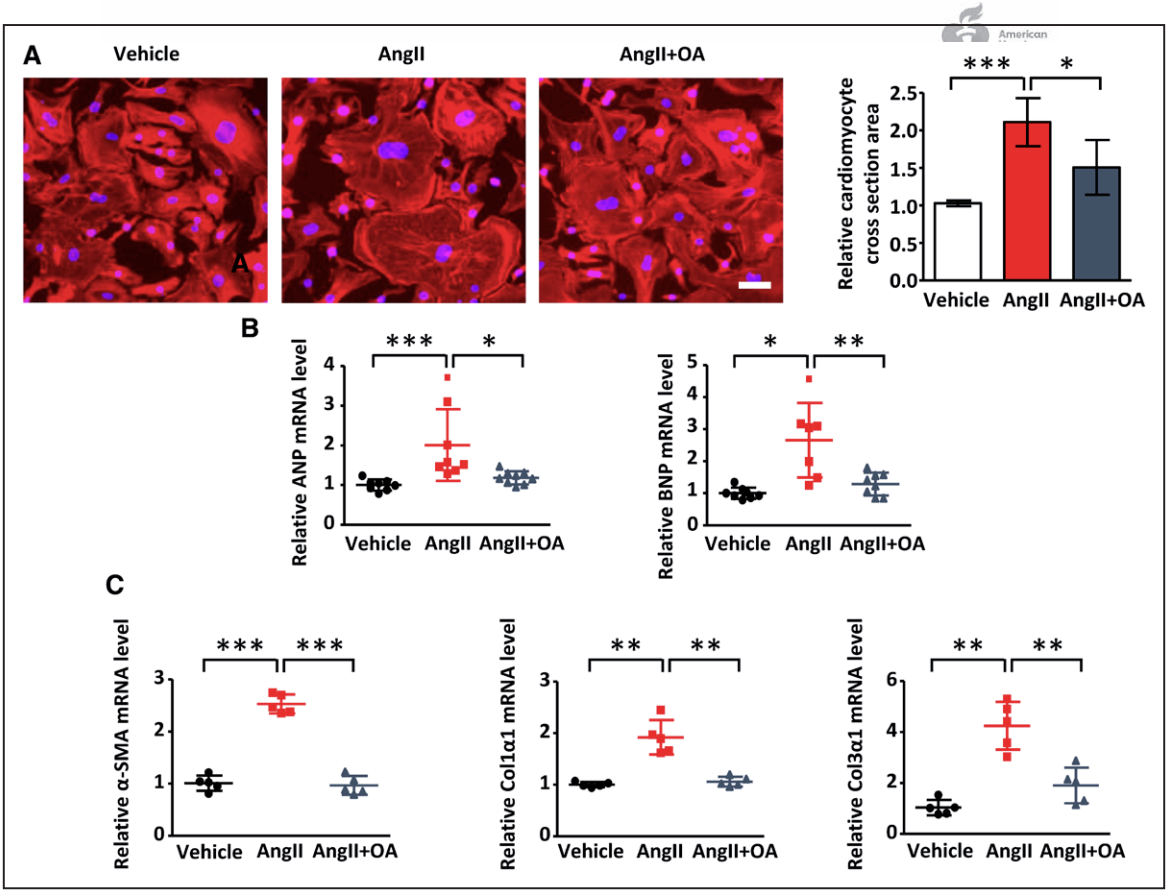

Figure 2. Oleic acid (OA) inhibited Ang II (Angiotensin II)-induced cardiomyocytes growth and cardiac fibroblasts collagen expression in vitro. **A**, Representative images of primary cardiomyocytes induced with Ang II and treated with OA for 48 hours, staining of the cytoskeleton with fluorescently labeled phalloidin to detect cell size, and quantification data of the cell area (Scale bar, 50 μm, n=5 independent experiments). **B**, ANP (atrial natriuretic peptide, n=8 independent experiments) and BNP (brain natriuretic peptide, n=7 independent experiments) mRNA level in neonatal mouse cardiomyocytes induced with Ang II and treated with OA for 24 hours. **C**, α-SMA (α-smooth muscle actin, n=5 independent experiments), Col1α1 (n=5 independent experiments), and Col3α1 (n=5 independent experiments) mRNA levels in Ang II-induced and OA-treated primary cardiomyocytes. Data are presented as the mean±SD, 1-way ANOVA with Tukey post hoc test or the Games-Howell post hoc test, **P*<0.05, ***P*<0.01, ****P*<0.001.

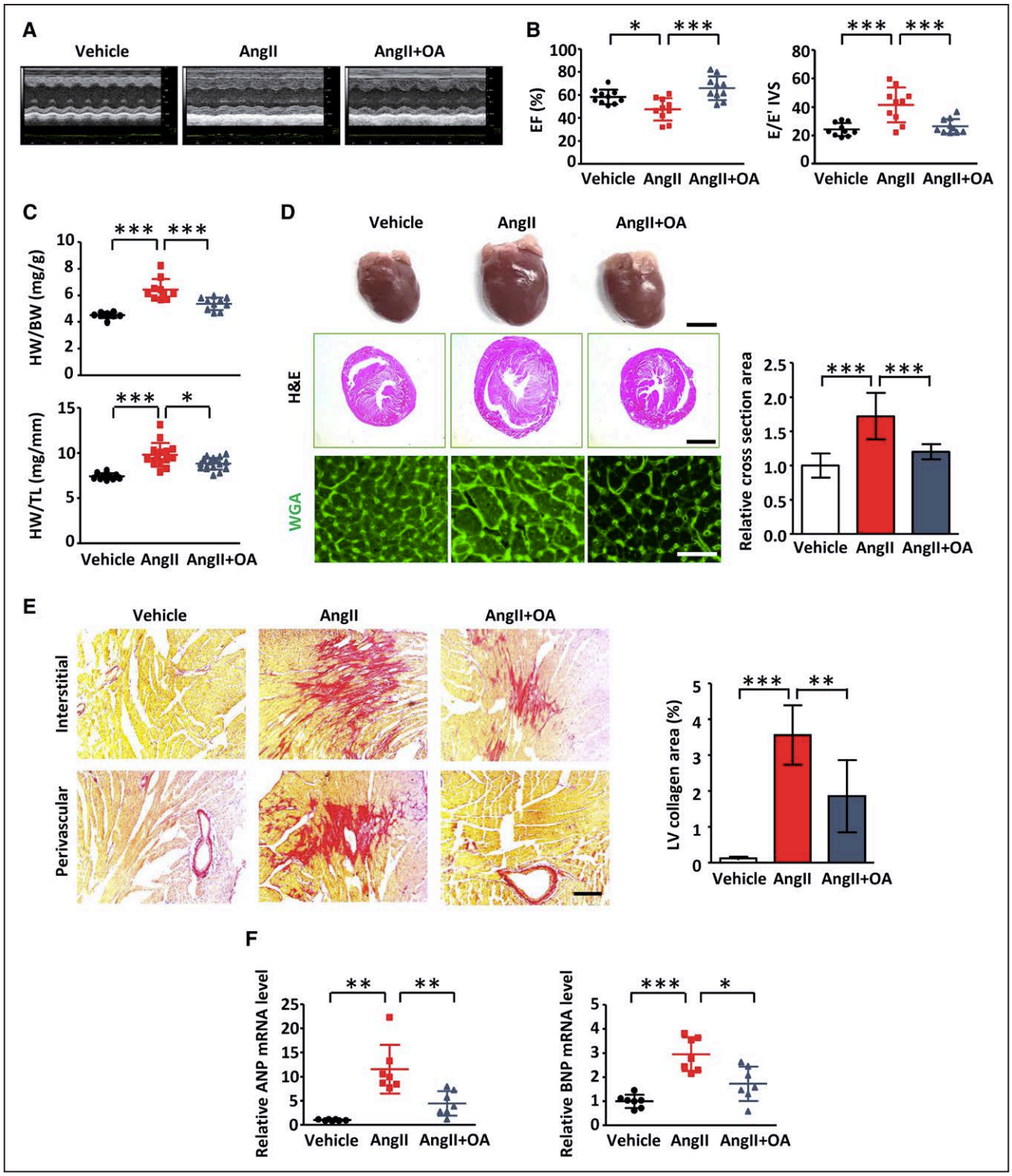

Figure 3. Oleic acid (OA) protects against Ang II (Angiotensin II)-induced cardiac remodeling in vivo. **A**, Representative M-mode echocardiographic tracings of different groups. **B**, Cardiac function was assessed by ejection fraction (EF%; n=10 per group) and E/E' IVS (n=10 per group). **C**, Heart weight to body weight ratio (n=10 per group) and heart weight to tibia length ratio (n=10 per group) of different groups. **D**, Representative images of the gross hearts (the first, scale bar, 0.5 cm), hematoxylin and eosin-stained (H&E, the second, scale bar, 2 mm), and wheat germ agglutinin-stained (the third, scale bar, 50 μm) transverse sections of the left ventricles from the indicated groups (n=6 per group). **E**, Picrosirius red-stained transverse sections of the left ventricles from the indicated groups. Scale bar, 200 μm (n=6 per group). **F**, ANP (atrial natriuretic peptide) and BNP (brain natriuretic peptide) mRNA levels in the hearts of the different groups (n=7 per group). Data are presented as the mean±SD, 1-way ANOVA with Tukey post hoc test or the Games-Howell post hoc test, **P*<0.05, ***P*<0.01, ****P*<0.001.

S5). After Ang II infusion for 2 weeks, the heart weight to body weight (HW/BW) and heart weight to tibia length (HW/TL) ratios were dramatically higher in Ang II-induced mice than in vehicle mice, and these increases were attenuated in OA-treated mice (Figure 3C). Consistently, both macroscopic and microscopic examinations of cardiac histology

revealed compromised cardiomyocyte hypertrophy in Ang II-induced mice, which was significantly attenuated in OA-treated mice (Figure 3D). Quantification of individual cardiomyocyte cross-sectional areas confirmed the prevention of hypertrophic growth in OA-treated mice (Figure 3D). OA-treated mice also displayed less collagen deposition than Ang II-induced mice, as visualized by picrosirius red staining (Figure 3E). Consistently, the expression of cardiac fetal genes (ANP and BNP was significantly augmented in Ang II-induced mice, while their expression was inhibited in OA-treated mice; Figure 3F). Besides, we found that treatment with OA starting first after 1 week of Ang II injection can also inhibit cardiac remodeling in mice (Figure S4).

RNA Sequencing Highlights Biological Processes and Target Molecules Modulated by OA

To gain a greater understanding of the protective effect of OA on Ang II-induced cardiac remodeling, we performed genome-wide transcriptional profiling (Figure 4A). For RNA sequencing, fold changes >1.5 or <0.67 in gene expression among the different groups and P values <0.05 were considered statistically significant. Compared with the vehicle mice, 983 and 481 differentially expressed genes were significantly up- and down-regulated, respectively, in the hearts of Ang II-induced mice (Figure 4B). Among the 983 upregulated genes, the expression changes in 388 genes were significantly reversed in the hearts of the OA-treated mice (Table S6; Figure 4C). We searched the promoter sequence of these 388 genes (defined upstream by 3500 bases of exons as promoter sequence) in UCSC website (http://genome.ucsc.edu), then those promoter sequences were respectively uploaded to public databases (http://gene-regulation.com/pub/databases.html) to enrich transcription factor that could combine with promoter sequence. However, there is no enrichment of TCF23 in the promoter region of these 388 genes.

Gene ontology analysis of the 388 genes were performed in DAVID database.[33] The results revealed that many of the associated biological processes, including fatty acid metabolic process, negative regulation of hormone secretion, positive regulation of ERK1 and ERK2 cascades, and cellular response to leptin stimulus, were closely associated with cardiac remodeling or cardiac hypertrophy (Figure 4D). The gene or protein most often involved in the biological processes enriched among the 388 differentially expressed genes was FGF23. Moreover, we verified the altered expression of FGF23 by q-PCR and ELISA in vivo (Figure 4E). Therefore, we considered that the protective effect of OA on Ang II-induced cardiac remodeling was related to the inhibition of FGF23 expression.

Systemic FGF23 Overexpression Aggravated Ang II-Induced Cardiac Remodeling and Weakened the Protective Effect of OA

We then assessed in vivo whether exogenous FGF23 could augment Ang II-induced cardiac remodeling and reverse the protective effect of OA. AAV9-FGF23 and AAV9-GFP vectors were intravenously injected 3 weeks before OA treatment and Ang II infusion in wild-type mice.[34] Overexpression of FGF23 in whole heart tissue was verified by fluorescence microscopy and Western blotting (Figures S5 and S6). Ang II-induced mice had a significantly higher systolic blood pressure than mice that did not receive Ang II infusion after the pump was implanted for 1 week. AAV9 injection and OA treatment had no effect on mouse systolic blood pressure during the experimental period (Figure S7).

We also assessed cardiac performance by echocardiography in Ad-GFP and Ad-FGF23 mice. No significant differences between the vehicle groups were observed. Both Ad-GFP and Ad-FGF23 mice developed systolic and diastolic dysfunction in response to Ang II, indicated by decreased EF and increased E/E', and these dysfunctions were attenuated by OA treatment. EF was significantly decreased and E/E' was significantly increased in Ang II-induced Ad-FGF23 mice compared with those in Ad-GFP mice, indicating a greater severity of cardiac dysfunction in Ad-FGF23 mice in response to pressure overload. Furthermore, overexpression of FGF23 attenuated the protective role of OA on cardiac remodeling, as evidenced by a significantly decreased ejection fraction and an increased E/E' in the hearts of OA-treated Ad-FGF23 mice compared with those of Ad-GFP mice (Figure 5A; Figure S8; Table S7).

Both Ad-FGF23 and Ad-GFP mice treated with OA displayed reduced cardiac hypertrophy in response to Ang II, whether in macroscopic or microscopic morphology, compared with Ang II-induced mice. However, overexpression of FGF23 significantly attenuated the protective effect of OA (Figure S9A), which was illustrated by a significant increase in the heart weight to body weight ratio (Figure S9B), heart weight to tibia length ratio, and individual cardiomyocyte cross-sectional area in OA-treated Ad-FGF23 mice compared with those in Ad-GFP mice (Figure 5B and 5C). Furthermore, compared with Ang II-induced Ad-GFP mice, Ad-FGF23 mice exhibited more severe cardiac hypertrophy.

FGF23 overexpression aggravated cardiac fibrosis as visualized by picrosirius red staining in Ang II-induced Ad-FGF23 mice and significantly weakened the protective role of OA compared with that observed in Ad-GFP mice. Greater collagen deposition was observed in the ventricular tissue of both Ang II-induced Ad-FGF23 mice and OA-treated Ad-FGF23 mice than in Ad-GFP mice (Figure 5D). Similar results were also observed for the cardiac ANP expression levels in mice in the different treatment groups. However, no significant difference in BNP mRNA levels was observed between Ad-FGF23 mice and Ad-GFP mice (Figure 5E).

The hearts of both Ang II-induced Ad-FGF23 mice and Ad-GFP mice exhibited substantially increased PLCγ (phosphoinositide-specific phospholipase Cγ)-NFAT1 (Nuclear factor of activated T cells) and TGF (transforming growth factor)-β/β-Catenin pathway activation, while this activation was attenuated in the hearts of the mice in the OA treatment group. Significantly decreased protein levels of TGF-β, β-Catenin, NFAT1, and PLCγ were observed in the hearts of OA-treated mice compared with those in the hearts of Ang II-induced mice. However, the PLCγ-NFAT1 pathway and TGF-β/β-Catenin pathway were enhanced by overexpression of FGF23 in the hearts of Ang II-induced Ad-FGF23 mice compared with those of Ang II-induced Ad-GFP mice. Moreover, the inhibitory effect

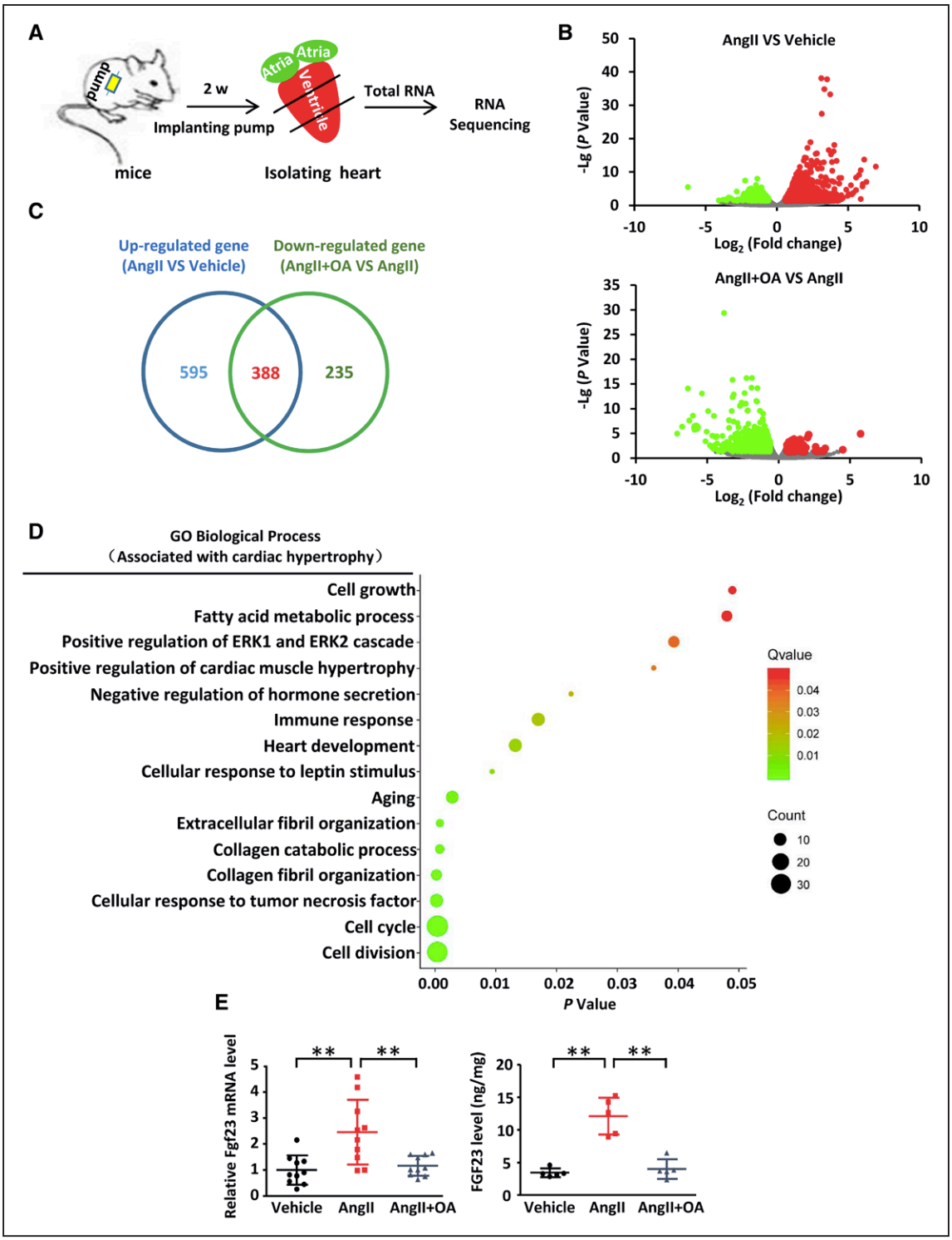

Figure 4. RNA sequencing highlights biological processes and target molecules modulated by oleic acid (OA). **A**, Total RNA of the ventricle was extracted and sequenced after implantation of an Ang II (Angiotensin II) pump for 2 wk. **B**, Volcano plot visualizing differential expression of the genes of the heart transcriptome. **C**, An overlap was performed between upregulated genes in the hearts of Ang II-induced mice and downregulated genes in the hearts of OA-treated mice. **D**, Gene ontology (GO) analysis results of 388 genes. **E**, RNA sequencing results were validated by q-PCR (n=10 per group) and ELISA (n=5 per group). Data are presented as the mean±SD, 1-way ANOVA with Tukey post hoc test, or the Games-Howell post hoc test, $^{**}P<0.01$.

of OA on the above pathways was weakened by overexpression of FGF23, as evidenced by significantly increased protein levels of PLCγ, TGF-β, and β-Catenin in the hearts of OA-treated Ad-FGF23 mice compared with those in OA-treated Ad-GFP mice (Figure 5F).

OA Suppressed Ang II-Induced FGF23 Expression by Inhibiting Translocation of Nurr1 From the Cytoplasm to the Nucleus

To analyze the potential effects of OA on FGF23 expression, we first performed q-PCR for FGF23 in Ang II-induced and

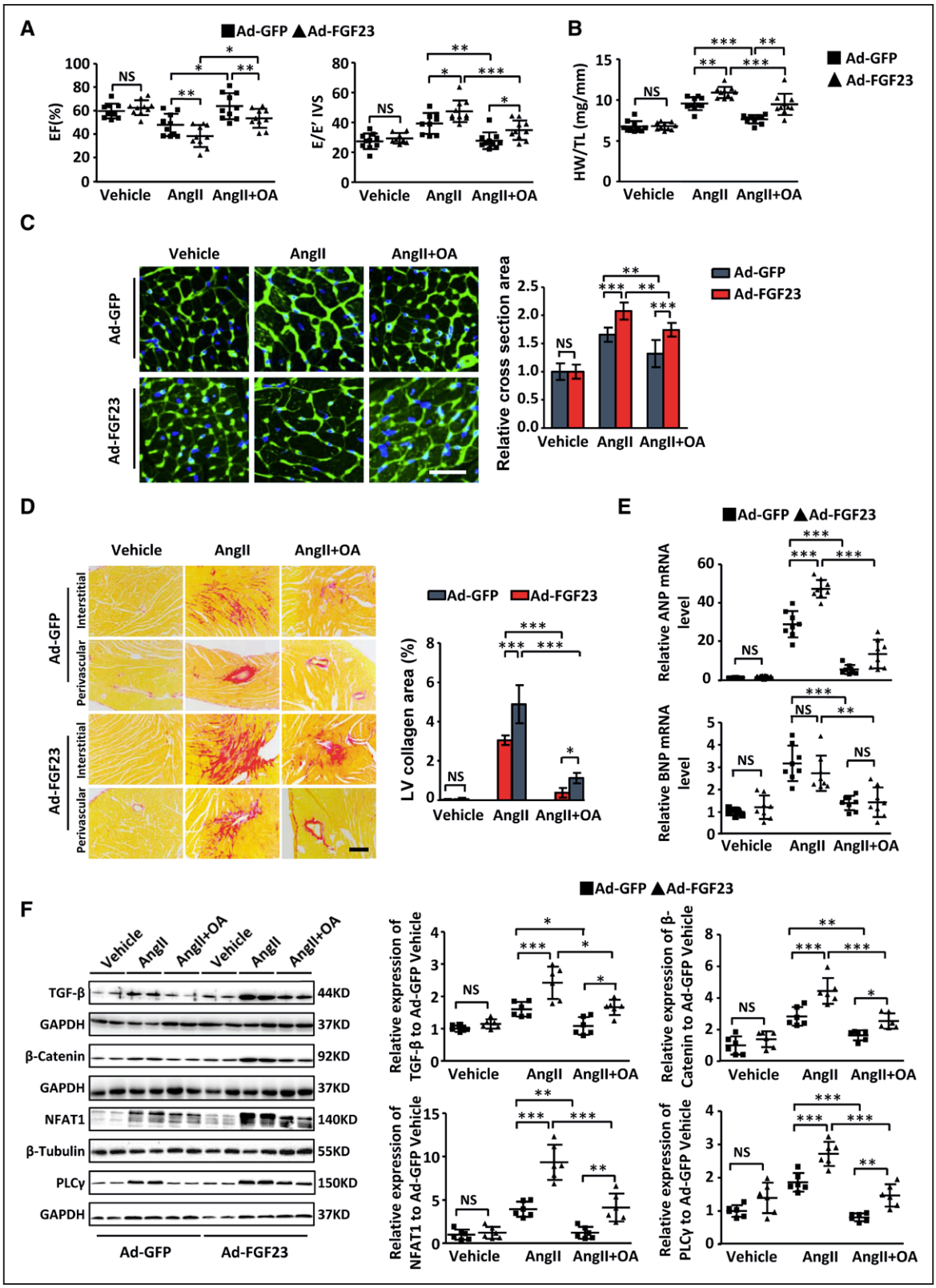

Figure 5. Overexpression of FGF23 (Fibroblast Growth Factor 23) aggravated Ang II (Angiotensin II)-induced cardiac remodeling and attenuated the protective effect of oleic acid (OA) on cardiac remodeling. **A**, Cardiac function was assessed by EF(%; n=10 per group) and E/E' IVS (n=8–10 per group). **B**, Heart weight to tibia length ratio (n=10 per group) in mice after Ang II pump implantation for 2 wks. **C**, wheat germ agglutinin staining to assess cardiomyocyte size and quantification of the cardiomyocyte size in different groups (Scale bar, 50 μm, n=6 per group). **D**, Sirius red staining to assess cardiac fibrosis and quantify cardiac fibrosis size in different groups (Scale bar, 50 μm, n=6 per group). **E**, ANP (atrial natriuretic peptide) and BNP (brain natriuretic peptide) mRNA levels in the hearts of different groups (n=8 per group). **F**, TGF-β (transforming growth factor-β), β-Catenin, NFAT1, and PLCγ (Phosphoinositide-specific phospholipase Cγ) expression levels in the heart detected by Western blotting and quantification of the expression levels in different groups (n=6 per group). Data are presented as the mean±SD, 2-way ANOVA with Bonferroni post hoc test. **P*<0.05, ***P*<0.01, ****P*<0.001. NS indicates no significance.

OA-treated cardiomyocytes and CFs. The results showed that OA suppressed the Ang II-induced expression of FGF23 in cardiomyocytes, which was not found in CFs (Figure S10). A previous study showed that the transcription factor Nurr1 (nuclear receptor–related 1 protein) can bind and transactivate FGF23 promoters by binding to cis elements to increase FGF23 expression.[35,36] Therefore, we detected Nurr1 expression levels to illustrate whether the protective effect of OA on Ang II-induced cardiac remodeling was associated with Nurr1 activity or expression in vitro. Our results showed that Ang II stimulation and OA treatment did not affect basal Nurr1 expression (Figure S11). However, the distribution of Nurr1 in the cytoplasm and nucleus differed significantly between Ang II-induced cardiomyocytes and OA-treated cardiomyocytes. Ang II could induce the translocation of Nurr1 from the cytoplasm to the nucleus to promote an increase in endogenous FGF23 expression in cardiomyocytes, whereas translocation was suppressed in OA-treated cardiomyocytes (Figure 6A and 6B). Identical results were obtained by immunofluorescence staining of cardiomyocytes (Figure 6C). Moreover, a dose-dependent effect of OA on Ang II-induced Nurr1 nuclear translocation was performed in cardiac myocytes. Our results showed that OA could dose-dependently inhibit Ang II-induced translocation of Nurr1 from the cytoplasm to the nucleus (Figure S12). Therefore, we concluded that OA suppressed Ang II-induced FGF23 transcription by inhibiting the translocation of Nurr1 from the cytoplasm to the nucleus.

Discussion

In the present study, we found decreased levels of OA in the peripheral blood of patients with hypertension with LVH compared with patients with hypertension without LVH. We speculated that OA plays a crucial role in the pathological process from hypertension to hypertensive cardiac remodeling. We further discovered that OA supplementation was able to prevent pathological cardiac remodeling by inhibiting FGF23 expression in the heart. Overexpression of FGF23 augmented cardiac hypertrophy and fibrosis and weakened OA function in mice following Ang II administration and OA treatment.

Changes in circulating and cardiac metabolites, which are the substrates or products of metabolic pathways or serve as regulatory signals with hormone-like functions, affected the key features of pathological cardiac remodeling, including hypertrophy and fibrosis.[37,38] We here demonstrate that the levels of 14 metabolites in the peripheral blood of patients with cardiac remodeling showed statistically significant differences compared with those in patients with hypertension, including OA. Similar to our results, the OA level was significantly decreased in cardiac tissue from 8-week-old double transgenic rats harboring human renin and angiotensinogen genes.[39] Those results suggested downregulation of fatty acid synthesis in patients with cardiac remodeling.[40] Indeed, a distinct pattern in the expression profile of genes encoding mitochondrial respiratory chain and lipid metabolism was found in a rat model of heart failure.[41] Those findings could also partially provide a basis for explaining the beneficial effects of OA supplementation in a mouse model of cardiac remodeling.

The pathological process of cardiac remodeling was associated with reduced contractile function in parallel with a shift in energy metabolism from fatty acids to glucose and a gradual decline of mitochondrial oxidative phosphorylation.[42,43] OA, as a primary monounsaturated fatty acid, exerts its biological effects primarily through activation of nuclear receptor PPARα (peroxisome proliferator-activated receptor-α), and then, activated PPARα binds to PPAR response elements to regulate the expression of a large number of genes involved in lipid transport, oxidation, lipogenesis, and lipoprotein uptake.[16,44] In the transcriptional data of mouse heart from our study, however, we did not find a significant difference in PPARα expression and PPARα target genes related to triglyceride turnover between the hearts of Ang II-induced mice and OA-treated mice, including carnitine palmitoyltransferase 1b (*Cpt1b*) and medium-chain acyl-CoA dehydrogenase (*Acadm*). By contrast, pyruvate dehydrogenase kinase 4 (*Pdhk4*) and other PPARα target genes related to triglyceride turnover were significantly decreased in the hearts of OA-treated mice compared with Ang II-induced mice (Figure S13).[45] In addition, Brian N. F's study showed that cardiac-restricted overexpression of PPARα did not inhibit the development of diabetic ventricular hypertrophy by regulating energy metabolism; conversely, PPARα could activate extramitochondrial lipid metabolic pathways, leading to the generation of reactive oxygen species and exacerbation of cardiomyopathy.[46] Therefore, we speculated that signaling pathways other than the PPARα signaling pathway were involved in the protective effect of OA on Ang II-induced cardiac remodeling. Here, an important finding of our study is that OA protected the heart from hypertrophy and fibrosis in response to Ang II through suppression of FGF23 expression.

FGF23 itself is known to induce cardiac remodeling in patients with chronic kidney disease.[47,48] Previous studies showed that FGF23 binding to fibroblast growth factor receptor 4 in cardiomyocytes resulted in cardiac remodeling through activation of α-klotho-independent PLCγ-NFAT1 signaling.[49,50] We found an increased level of FGF23 in murine cardiac tissue in response to Ang II infusion; moreover, the increased FGF23 was mainly from cardiomyocytes. FGF23 binding to its receptor led to phosphorylation of PLCγ (phospholipase Cγ), which promoted expression of numerous transcription factors via the Ca^{2+}-dependent pathway, including NFAT1, MEF2, and SRF, and those factors have a close relationship with cardiac integrity, pathological remodeling, and cardiac hypertrophy.[51,52] We found that OA was able to suppress FGF23 expression and PLCγ-NFAT1 signaling activation in the heart in response to Ang II infusion, although this was weakened by overexpression of FGF23.

TGF (transforming growth factor)-β is one of the most common cytokines associated with Ang II or overload-induced pathological cardiac fibrosis. Previous studies have shown that Ang II and TGF-β could promote cardiac fibrosis and fibrotic gene expression via activating Wnt/β-Catenin signaling.[53,54] In vitro, ECM (Extracellular matrix)

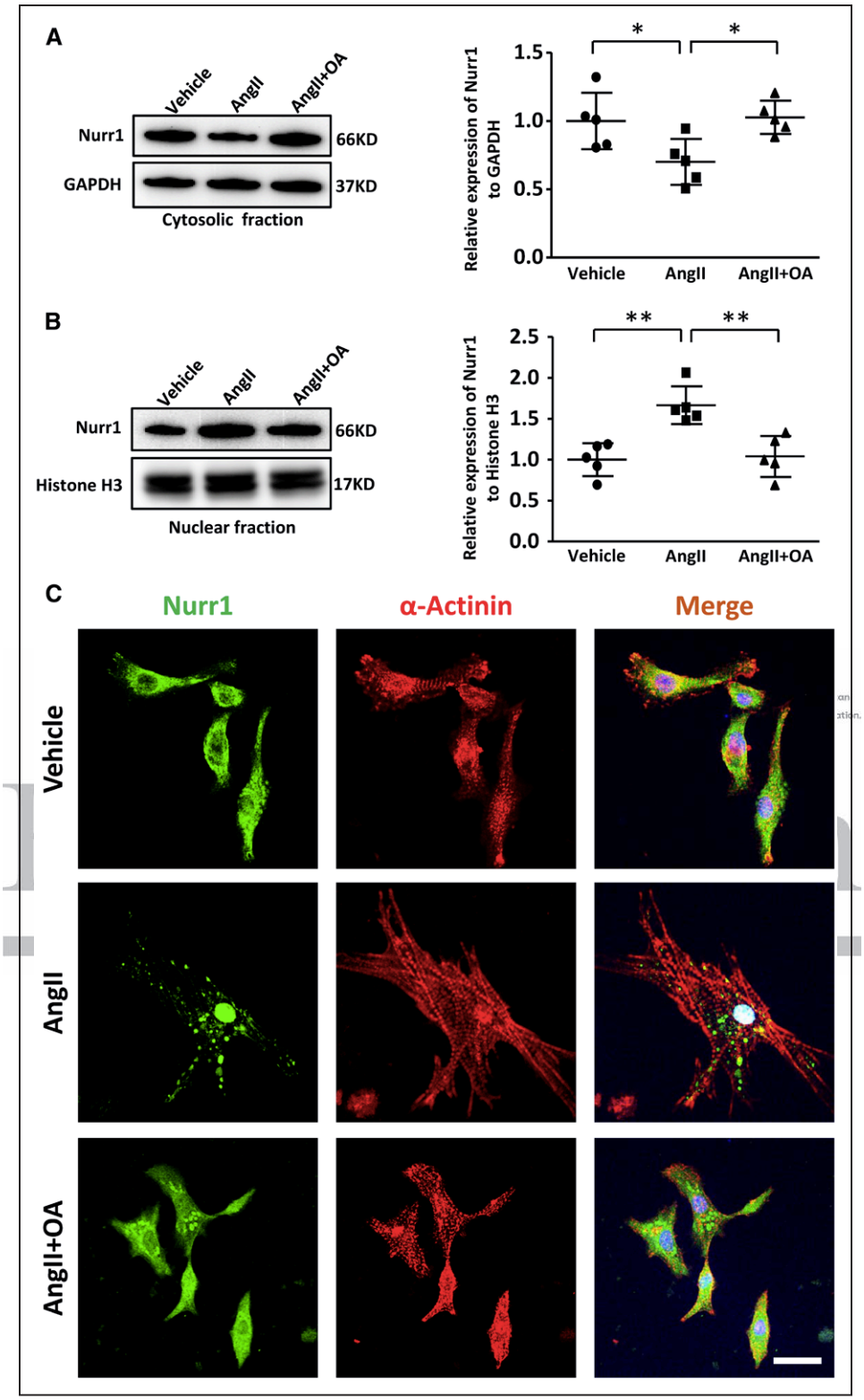

Figure 6. Oleic acid (OA) suppressed Ang II (Angiotensin II)-induced FGF23 (Fibroblast Growth Factor 23) expression by inhibiting the translocation of Nurr1 (nuclear receptor–related 1 protein) from the cytoplasm to the nucleus. **A**, The expression level of Nurr1 in the cytosolic fraction (n=5 independent experiments). **B**, The expression level of Nurr1 in the nuclear fraction (n=5 independent experiments). **C**, Immunofluorescence was used to stain Nurr1 in CMs (Scale bar, 20 μm). Data are presented as the mean±SD, 1-way ANOVA with Tukey post hoc test, or the Games-Howell post hoc test, **P*<0.05, ***P*<0.01.

gene expression and ECM deposition were directly induced by TGF-β in CFs, which was achieved by suppressing matrix metalloproteinase expression and simultaneously inducing tissue inhibitors of matrix metalloproteinase gene expression.[55] We also found that TGF-β regulated cardiac fibrosis in response to Ang II or FGF23 in vivo and in vitro, while those effects were suppressed by OA supplementation. Moreover, β-Catenin as a subunit of the cell surface

cadherin protein complex was a critical downstream regulator of TGF-β signaling.[53] Activated β-Catenin produced by TGF-β signaling translocates into the nucleus and binds to T cell factor/lymphoid enhancer-binding factor to stimulate the transcription of target genes associated with fibrosis.[56] Indeed, increased protein levels of β-Catenin and TGF-β were found in the heart in response to Ang II, although the increases were significantly inhibited by OA supplementation.

The transcription factor Nurr1 is a member of a subfamily of orphan nuclear receptors with no identified ligand.[57] A previous study showed that Nurr1 plays a prominent role in regulating inflammation reactions,[58] vascular cell function,[59] stress, and addiction.[60] Stephen A. Myers's study showed that Nurr1 mRNA levels were significantly upregulated by β-adrenergic-induced cardiac remodeling in mouse heart.[61] However, the mechanism of action of Nurr1 in cardiac hypertrophy and heart failure remains unclear. Our study is the first to show that Ang II-induced cardiac remodeling is associated with increased translocation of Nurr1 from the cytoplasm to the nucleus to facilitate FGF23 transcription, this process was blunted by OA treatment.

Perspectives

In the present study, a significantly decreased oleic acid levels were observed in the peripheral blood of patients with hypertension with LVH by comparing plasma metabolic character between patients with hypertension with and without LVH, and then the protective effect of OA supplementation on pathological cardiac remodeling was validated in vivo and in vitro. Our study demonstrates for the first time that OA supplementation prevented hypertensive cardiac remodeling by inhibiting Nurr1-FGF23 signaling. Therefore, OA supplementation may be considered as potential prevention strategy for hypertensive cardiac remodeling.

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Disclosures

None.

References

1. Lu J, Lu Y, Wang X, Li X, Linderman GC, Wu C, Cheng X, Mu L, Zhang H, Liu J, et al. Prevalence, awareness, treatment, and control of hypertension in China: data from 1·7 million adults in a population-based screening study (China PEACE Million Persons Project). *Lancet.* 2017;390:2549–2558. doi: 10.1016/S0140-6736(17)32478-9
2. Lam CS, Teng TK, Tay WT, Anand I, Zhang S, Shimizu W, Narasimhan C, Park SW, Yu CM, Ngarmukos T, et al. Regional and ethnic differences among patients with heart failure in Asia: the Asian sudden cardiac death in heart failure registry. *Eur Heart J.* 2016;37:3141–3153. doi: 10.1093/eurheartj/ehw331
3. Xiao H, Li H, Wang JJ, Zhang JS, Shen J, An XB, Zhang CC, Wu JM, Song Y, Wang XY, et al. IL-18 cleavage triggers cardiac inflammation and fibrosis upon β-adrenergic insult. *Eur Heart J.* 2018;39:60–69. doi: 10.1093/eurheartj/ehx261
4. González A, López B, Ravassa S, Beaumont J, Arias T, Hermida N, Zudaire A, Díez J. Biochemical markers of myocardial remodelling in hypertensive heart disease. *Cardiovasc Res.* 2009;81:509–518. doi: 10.1093/cvr/cvn235
5. Mohamed TMA, Abou-Leisa R, Stafford N, Maqsood A, Zi M, Prehar S, Baudoin-Stanley F, Wang X, Neyses L, Cartwright EJ, et al. The plasma membrane calcium ATPase 4 signalling in cardiac fibroblasts mediates cardiomyocyte hypertrophy. *Nat Commun.* 2016;7:11074. doi: 10.1038/ncomms11074
6. Monserrat L, López B, González A, Hermida M, Fernández X, Ortiz M, Barriales-Villa R, Castro-Beiras A, Díez J. Cardiotrophin-1 plasma levels are associated with the severity of hypertrophy in hypertrophic cardiomyopathy. *Eur Heart J.* 2011;32:177–183. doi: 10.1093/eurheartj/ehq400
7. Zile MR, Desantis SM, Baicu CF, Stroud RE, Thompson SB, McClure CD, Mehurg SM, Spinale FG. Plasma biomarkers that reflect determinants of matrix composition identify the presence of left ventricular hypertrophy and diastolic heart failure. *Circ Heart Fail.* 2011;4:246–256. doi: 10.1161/CIRCHEARTFAILURE.110.958199
8. Roncarati R, Viviani Anselmi C, Losi MA, Papa L, Cavarretta E, Da Costa Martins P, Contaldi C, Saccani Jotti G, Franzone A, Galastri L, et al. Circulating miR-29a, among other up-regulated microRNAs, is the only biomarker for both hypertrophy and fibrosis in patients with hypertrophic cardiomyopathy. *J Am Coll Cardiol.* 2014;63:920–927. doi: 10.1016/j.jacc.2013.09.041
9. Hoefer IE, Steffens S, Ala-Korpela M, Bäck M, Badimon L, Bochaton-Piallat ML, Boulanger CM, Caligiuri G, Dimmeler S, Egido J, et al.; ESC Working Group Atherosclerosis and Vascular Biology. Novel methodologies for biomarker discovery in atherosclerosis. *Eur Heart J.* 2015;36:2635–2642. doi: 10.1093/eurheartj/ehv236
10. Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, Lewis GD, Fox CS, Jacques PF, Fernandez C, et al. Metabolite profiles and the risk of developing diabetes. *Nat Med.* 2011;17:448–453. doi: 10.1038/nm.2307
11. Sa R, Zhang W, Ge J, Wei X, Zhou Y, Landzberg DR, Wang Z, Han X, Chen L, Yin H. Discovering a critical transition state from nonalcoholic hepatosteatosis to nonalcoholic steatohepatitis by lipidomics and dynamical network biomarkers. *J Mol Cell Biol.* 2016;8:195–206. doi: 10.1093/jmcb/mjw016
12. Li M, Zeng T, Liu R, Chen L. Detecting tissue-specific early warning signals for complex diseases based on dynamical network biomarkers: study of type 2 diabetes by cross-tissue analysis. *Brief Bioinform.* 2014;15:229–243. doi: 10.1093/bib/bbt027
13. Fan Y, Li Y, Chen Y, Zhao YJ, Liu LW, Li J, Wang SL, Alolga RN, Yin Y, Wang XM, et al. Comprehensive Metabolomic Characterization of Coronary Artery Diseases. *J Am Coll Cardiol.* 2016;68:1281–1293. doi: 10.1016/j.jacc.2016.06.044
14. Bernardo BC, Weeks KL, Pretorius L, McMullen JR. Molecular distinction between physiological and pathological cardiac hypertrophy: experimental findings and therapeutic strategies. *Pharmacol Ther.* 2010;128:191–227. doi: 10.1016/j.pharmthera.2010.04.005
15. Brookes PS, Taegtmeyer H. Metabolism: A Direct Link Between Cardiac Structure and Function. *Circulation.* 2017;136:2158–2161. doi: 10.1161/CIRCULATIONAHA.117.031372
16. Bowen KJ, Kris-Etherton PM, Shearer GC, West SG, Reddivari L, Jones PJH. Oleic acid-derived oleoylethanolamide: A nutritional science perspective. *Prog Lipid Res.* 2017;67:1–15. doi: 10.1016/j.plipres.2017.04.001
17. Goncalves de Albuquerque CF, Burth P, Younes Ibrahim M, Garcia DG, Bozza PT, Castro Faria Neto HC, Castro Faria MV. Reduced plasma non-esterified fatty acid levels and the advent of an acute lung injury in mice after intravenous or enteral oleic acid administration. *Mediators Inflamm.* 2012;2012:601032
18. Hernáez Á, Castañer O, Elosua R, Pintó X, Estruch R, Salas-Salvadó J, Corella D, Arós F, Serra-Majem L, Fiol M, et al. Mediterranean Diet Improves High-Density Lipoprotein Function in High-Cardiovascular-Risk Individuals: A Randomized Controlled Trial. *Circulation.* 2017;135:633–643. doi: 10.1161/CIRCULATIONAHA.116.023712
19. Bloomfield HE, Koeller E, Greer N, MacDonald R, Kane R, Wilt TJ. Effects on Health Outcomes of a Mediterranean Diet With No Restriction on Fat Intake: A Systematic Review and Meta-analysis. *Ann Intern Med.* 2016;165:491–500. doi: 10.7326/M16-0361
20. Tektonidis TG, Åkesson A, Gigante B, Wolk A, Larsson SC. A Mediterranean diet and risk of myocardial infarction, heart failure and stroke: A population-based cohort study. *Atherosclerosis.* 2015;243:93–98. doi: 10.1016/j.atherosclerosis.2015.08.039
21. Clara R, Langhans W, Mansouri A. Oleic acid stimulates glucagon-like peptide-1 release from enteroendocrine cells by modulating cell

respiration and glycolysis. *Metabolism.* 2016;65:8–17. doi: 10.1016/j.metabol.2015.10.003
22. Lin YK, Chen YC, Kao YH, Tsai CF, Yeh YH, Huang JL, Cheng CC, Chen SA, Chen YJ. A monounsaturated fatty acid (oleic acid) modulates electrical activity in atrial myocytes with calcium and sodium dysregulation. *Int J Cardiol.* 2014;176:191–198. doi: 10.1016/j.ijcard.2014.07.004
23. Song WH, Lin YH, Sun K, Zhang YH, Song Y, Hou LB, Zhang CN, Hui RT, Chen JZ. A functional variant in the coding region of CAMTA2 is associated with left ventricular hypertrophy by affecting the activation of Nkx2.5-dependent transcription. *J Hypertens.* 2016;34:942–949. doi: 10.1097/HJH.0000000000000873
24. Li H, Xu H, Wen H, Liu T, Sun Y, Xiao N, Bai C, Ge J, Wang X, Song L, et al. Overexpression of LH3 reduces the incidence of hypertensive intracerebral hemorrhage in mice. *J Cereb Blood Flow Metab.* 2019;39:547–561. doi: 10.1177/0271678X18815791
25. Fan D, Takawale A, Basu R, Patel V, Lee J, Kandalam V, Wang X, Oudit GY, Kassiri Z. Differential role of TIMP2 and TIMP3 in cardiac hypertrophy, fibrosis, and diastolic dysfunction. *Cardiovasc Res.* 2014;103:268–280. doi: 10.1093/cvr/cvu072
26. Monteiro M, Moreira N, Pinto J, Pires-Luís AS, Henrique R, Jerónimo C, Bastos ML, Gil AM, Carvalho M, Guedes de Pinho P. GC-MS metabolomics-based approach for the identification of a potential VOC-biomarker panel in the urine of renal cell carcinoma patients. *J Cell Mol Med.* 2017;21:2092–2105. doi: 10.1111/jcmm.13132
27. Luo P, Yin P, Hua R, Tan Y, Li Z, Qiu G, Yin Z, Xie X, Wang X, Chen W, et al. A Large-scale, multicenter serum metabolite biomarker identification study for the early detection of hepatocellular carcinoma. *Hepatology.* 2018;67:662–675. doi: 10.1002/hep.29561
28. Arbel H, Basu S, Fisher WW, Hammonds AS, Wan KH, Park S, Weiszmann R, Booth BW, Keranen SV, Henriquez C, et al. Exploiting regulatory heterogeneity to systematically identify enhancers with high accuracy. *Proc Natl Acad Sci U S A.* 2019;116:900–908. doi: 10.1073/pnas.1808833115
29. Nagai T, Anzai T, Mano Y, Kaneko H, Anzai A, Sugano Y, Maekawa Y, Takahashi T, Yoshikawa T, Fukuda K. Eicosapentaenoic acid suppresses adverse effects of C-reactive protein overexpression on pressure overload-induced cardiac remodeling. *Heart Vessels.* 2013;28:404–411. doi: 10.1007/s00380-012-0270-5
30. Siddiqui RA, Shaikh SR, Kovacs R, Stillwell W, Zaloga G. Inhibition of phenylephrine-induced cardiac hypertrophy by docosahexaenoic acid. *J Cell Biochem.* 2004;92:1141–1159. doi: 10.1002/jcb.20135
31. Fung TT, Rexrode KM, Mantzoros CS, Manson JE, Willett WC, Hu FB. Mediterranean diet and incidence of and mortality from coronary heart disease and stroke in women. *Circulation.* 2009;119:1093–1100. doi: 10.1161/CIRCULATIONAHA.108.816736
32. Martínez-González MA, Gea A, Ruiz-Canela M. The Mediterranean Diet and Cardiovascular Health. *Circ Res.* 2019;124:779–798. doi: 10.1161/CIRCRESAHA.118.313348
33. Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc.* 2009;4:44–57. doi: 10.1038/nprot.2008.211
34. Foust KD, Nurre E, Montgomery CL, Hernandez A, Chan CM, Kaspar BK. Intravascular AAV9 preferentially targets neonatal neurons and adult astrocytes. *Nat Biotechnol.* 2009;27:59–65. doi: 10.1038/nbt.1515
35. Wang Z, Benoit G, Liu J, Prasad S, Aarnisalo P, Liu X, Xu H, Walker NP, Perlmann T. Structure and function of Nurr1 identifies a class of ligand-independent nuclear receptors. *Nature.* 2003;423:555–560. doi: 10.1038/nature01645
36. Meir T, Durlacher K, Pan Z, Amir G, Richards WG, Silver J, Naveh-Many T. Parathyroid hormone activates the orphan nuclear receptor Nurr1 to induce FGF23 transcription. *Kidney Int.* 2014;86:1106–1115. doi: 10.1038/ki.2014.215
37. Lewis GD, Wei R, Liu E, Yang E, Shi X, Martinovic M, Farrell L, Asnani A, Cyrille M, Ramanathan A, et al. Metabolite profiling of blood from individuals undergoing planned myocardial infarction reveals early markers of myocardial injury. *J Clin Invest.* 2008;118:3503–3512. doi: 10.1172/JCI35111
38. Turer AT, Stevens RD, Bain JR, Muehlbauer MJ, van der Westhuizen J, Mathew JP, Schwinn DA, Glower DD, Newgard CB, Podgoreanu MV. Metabolomic profiling reveals distinct patterns of myocardial substrate use in humans with coronary artery disease or left ventricular dysfunction during surgical ischemia/reperfusion. *Circulation.* 2009;119:1736–1746. doi: 10.1161/CIRCULATIONAHA.108.816116
39. Mervaala E, Biala A, Merasto S, Lempiäinen J, Mattila I, Martonen E, Eriksson O, Louhelainen M, Finckenberg P, Kaheinen P, et al. Metabolomics in angiotensin II-induced cardiac hypertrophy. *Hypertension.* 2010;55:508–515. doi: 10.1161/HYPERTENSIONAHA.109.145490
40. Drosatos K, Schulze PC. Savings precede spending: fatty acid utilization relies on triglyceride formation for cardiac energetics. *Circulation.* 2014;130:1775–1777. doi: 10.1161/CIRCULATIONAHA.114.013048
41. Wellner M, Dechend R, Park JK, Shagdarsuren E, Al-Saadi N, Kirsch T, Gratze P, Schneider W, Meiners S, Fiebeler A, et al. Cardiac gene expression profile in rats with terminal heart failure and cachexia. *Physiol Genomics.* 2005;20:256–267. doi: 10.1152/physiolgenomics.00165.2004
42. Siddiqi N, Singh S, Beadle R, Dawson D, Frenneaux M. Cardiac metabolism in hypertrophy and heart failure: implications for therapy. *Heart Fail Rev.* 2013;18:595–606. doi: 10.1007/s10741-012-9359-2
43. Tuomainen T, Tavi P. The role of cardiac energy metabolism in cardiac hypertrophy and failure. *Exp Cell Res.* 2017;360:12–18. doi: 10.1016/j.yexcr.2017.03.052
44. Van Harken DR, Dixon CW, Heimberg M. Hepatic lipid metabolism in experimental diabetes. V. The effect of concentration of oleate on metabolism of triglycerides and on ketogenesis. *J Biol Chem.* 1969;244:2278–2285.
45. Lahey R, Wang X, Carley AN, Lewandowski ED. Dietary fat supply to failing hearts determines dynamic lipid signaling for nuclear receptor activation and oxidation of stored triglyceride. *Circulation.* 2014;130:1790–1799. doi: 10.1161/CIRCULATIONAHA.114.011687
46. Finck BN, Han X, Courtois M, Aimond F, Nerbonne JM, Kovacs A, Gross RW, Kelly DP. A critical role for PPARalpha-mediated lipotoxicity in the pathogenesis of diabetic cardiomyopathy: modulation by dietary fat content. *Proc Natl Acad Sci U S A.* 2003;100:1226–1231. doi: 10.1073/pnas.0336724100
47. Gutiérrez OM, Januzzi JL, Isakova T, Laliberte K, Smith K, Collerone G, Sarwar A, Hoffmann U, Coglianese E, Christenson R, et al. Fibroblast growth factor 23 and left ventricular hypertrophy in chronic kidney disease. *Circulation.* 2009;119:2545–2552. doi: 10.1161/CIRCULATIONAHA.108.844506
48. Ix JH, Katz R, Kestenbaum BR, de Boer IH, Chonchol M, Mukamal KJ, Rifkin D, Siscovick DS, Sarnak MJ, Shlipak MG. Fibroblast growth factor-23 and death, heart failure, and cardiovascular events in community-living individuals: CHS (Cardiovascular Health Study). *J Am Coll Cardiol.* 2012;60:200–207. doi: 10.1016/j.jacc.2012.03.040
49. Grabner A, Amaral AP, Schramm K, Singh S, Sloan A, Yanucil C, Li J, Shehadeh LA, Hare JM, David V, et al. Activation of Cardiac Fibroblast Growth Factor Receptor 4 Causes Left Ventricular Hypertrophy. *Cell Metab.* 2015;22:1020–1032. doi: 10.1016/j.cmet.2015.09.002
50. Touchberry CD, Green TM, Tchikrizov V, Mannix JE, Mao TF, Carney BW, Girgis M, Vincent RJ, Wetmore LA, Dawn B, et al. FGF23 is a novel regulator of intracellular calcium and cardiac contractility in addition to cardiac hypertrophy. *Am J Physiol Endocrinol Metab.* 2013;304:E863–E873. doi: 10.1152/ajpendo.00596.2012
51. Degirolamo C, Sabbà C, Moschetta A. Therapeutic potential of the endocrine fibroblast growth factors FGF19, FGF21 and FGF23. *Nat Rev Drug Discov.* 2016;15:51–69. doi: 10.1038/nrd.2015.9
52. Dewenter M, von der Lieth A, Katus HA, Backs J. Calcium Signaling and Transcriptional Regulation in Cardiomyocytes. *Circ Res.* 2017;121:1000–1020. doi: 10.1161/CIRCRESAHA.117.310355
53. Xiang FL, Fang M, Yutzey KE. Loss of β-catenin in resident cardiac fibroblasts attenuates fibrosis induced by pressure overload in mice. *Nat Commun.* 2017;8:712. doi: 10.1038/s41467-017-00840-w
54. Davis J, Molkentin JD. Myofibroblasts: trust your heart and let fate decide. *J Mol Cell Cardiol.* 2014;70:9–18. doi: 10.1016/j.yjmcc.2013.10.019
55. Leask A. Targeting the TGFbeta, endothelin-1 and CCN2 axis to combat fibrosis in scleroderma. *Cell Signal.* 2008;20:1409–1414. doi: 10.1016/j.cellsig.2008.01.006
56. Duan J, Gherghe C, Liu D, Hamlett E, Srikantha L, Rodgers L, Regan JN, Rojas M, Willis M, Leask A, et al. Wnt1/βcatenin injury response activates the epicardium and cardiac fibroblasts to promote cardiac repair. *EMBO J.* 2012;31:429–442. doi: 10.1038/emboj.2011.418
57. Jakaria M, Haque ME, Cho DY, Azam S, Kim IS, Choi DK. Molecular Insights into NR4A2(Nurr1): an Emerging Target for Neuroprotective Therapy Against Neuroinflammation and Neuronal Cell Death. *Mol Neurobiol.* 2019;56:5799–5814. doi: 10.1007/s12035-019-1487-4

58. Rodríguez-Calvo R, Tajes M, Vázquez-Carrera M. The NR4A subfamily of nuclear receptors: potential new therapeutic targets for the treatment of inflammatory diseases. *Expert Opin Ther Targets.* 2017;21:291–304. doi: 10.1080/14728222.2017.1279146
59. Martínez-González J, Badimon L. The NR4A subfamily of nuclear receptors: new early genes regulated by growth factors in vascular cells. *Cardiovasc Res.* 2005;65:609–618. doi: 10.1016/j.cardiores.2004.10.002
60. Campos-Melo D, Galleguillos D, Sánchez N, Gysling K, Andrés ME. Nur transcription factors in stress and addiction. *Front Mol Neurosci.* 2013;6:44. doi: 10.3389/fnmol.2013.00044
61. Myers SA, Eriksson N, Burow R, Wang SC, Muscat GE. Beta-adrenergic signaling regulates NR4A nuclear receptor and metabolic gene expression in multiple tissues. *Mol Cell Endocrinol.* 2009;309:101–108. doi: 10.1016/j.mce.2009.05.006

Novelty and Significance

What Is New?

- Plasma metabolic profiles were compared between patients with hypertension with and without left ventricular hypertrophy, and significantly decreased oleic acid levels were observed in the peripheral blood of patients with hypertension with left ventricular hypertrophy. Oleic acid supplementation prevented hypertensive cardiac remodeling by inhibiting Nurr1 (nuclear receptor–related 1 protein)-FGF23 (Fibroblast Growth Factor 23) signaling in the heart in vivo and in vitro.

What Is Relevant?

- Cardiac remodeling as a common hypertensive target organ damage is associated with arrhythmogenesis, heart failure, and mortality. However, few pharmacological therapies are currently available for the treatment of cardiac hypertrophy. Our data demonstrate that oleic acid can prevent AngII (Angiotensin II)-induced hypertensive cardiac remodeling by inhibiting Nurr1-FGF23 signaling. Therefore, oleic acid supplementation may be suggested as potential prevention strategy for hypertensive cardiac remodeling.

Summary

Oleic acid can prevent Ang II-induced cardiac remodeling via suppression of FGF23 expression, while this process is associated with the inhibitive effect of oleic acid on AngII-induced translocation of Nurr1 from the cytoplasm to the nucleus to facilitate FGF23 transcription.