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Original Paper

Deletion of miRNA-22 Induces Cardiac Hypertrophy in Females but Attenuates Obesogenic Diet-Mediated Metabolic Disorders

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Key Words
Obesity • miRNA-22 • Female • Cardiac hypertrophy • Metabolic dysfunction

Abstract
Background/Aims: Obesity is a risk factor associated with cardiometabolic complications. Recently, we reported that miRNA-22 deletion attenuated high-fat diet-induced adiposity and prevented dyslipidemia without affecting cardiac hypertrophy in male mice. In this study, we examined the impact of miRNA-22 in obesogenic diet-induced cardiovascular and metabolic disorders in females. ***Methods:*** Wild type (WT) and miRNA-22 knockout (miRNA-22 KO) females were fed a control or an obesogenic diet. Body weight gain, adiposity, glucose tolerance, insulin tolerance, and plasma levels of total cholesterol and triglycerides were measured. Cardiac and white adipose tissue remodeling was assessed by histological analyses. Echocardiography was used to evaluate cardiac function and morphology. RNA-sequencing analysis was employed to characterize mRNA expression profiles in female hearts. ***Results:*** Loss of miRNA-22 attenuated body weight gain, adiposity, and prevented obesogenic diet-

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induced insulin resistance and dyslipidemia in females. WT obese females developed cardiac hypertrophy. Interestingly, miRNA-22 KO females displayed cardiac hypertrophy without left ventricular dysfunction and myocardial fibrosis. Both miRNA-22 deletion and obesogenic diet changed mRNA expression profiles in female hearts. Enrichment analysis revealed that genes associated with regulation of the force of heart contraction, protein folding and fatty acid oxidation were enriched in hearts of WT obese females. In addition, genes related to thyroid hormone responses, heart growth and PI3K signaling were enriched in hearts of miRNA-22 KO females. Interestingly, miRNA-22 KO obese females exhibited reduced mRNA levels of *Yap1*, *Egfr* and *Tgfbr1* compared to their respective controls. ***Conclusion:*** This study reveals that miRNA-22 deletion induces cardiac hypertrophy in females without affecting myocardial function. In addition, our findings suggest miRNA-22 as a potential therapeutic target to treat obesity-related metabolic disorders in females.

Introduction

Obesity and being overweight are risk factors associated with several cardiovascular and metabolic disorders, such as dyslipidemia, type 2 diabetes, insulin resistance, hypertension and left ventricular hypertrophy [1-3]. Cardiovascular diseases are the leading cause of morbidity and death worldwide [4]. Obesity-related cardiac remodeling is mediated by a complex interaction between metabolic, inflammatory, neurohumoral and hemodynamic factors, resulting in cardiomyocyte hypertrophy and fibrosis, which may be associated with alterations in myocardial function [5, 6]. Some clinical and animal studies have revealed a relationship between visceral adipose tissue expansion and increased cardiometabolic risk [7]. In fact, the adipose tissue exerts a key role in cardiovascular health and disease due to its paracrine and endocrine actions [8].

Despite numerous efforts to characterize the biological mechanisms involved in obesity-related disorders, few studies have investigated the impact of sex differences in metabolic and cardiovascular complications associated with obesity. Men have an elevated risk for type 2 diabetes, insulin resistance and cardiovascular diseases compared with premenopausal women [9-11]. However, this difference in metabolic and cardiovascular diseases is reduced after menopause [12, 13]. Interestingly, diabetic obese women display cardiac hypertrophy and elevated risk of cardiovascular complications compared to diabetic obese men [14-17], suggesting that obesity reduces the protective effects of estrogens in the cardiovascular system.

MicroRNAs (miRNAs) are short non-coding RNAs involved in post-transcriptional regulation of gene expression by inhibiting translation and/or reducing mRNA stability. Several studies have reported the involvement of miRNAs in control of many biological processes, such as differentiation, metabolism, apoptosis and inflammation, which influence diverse physiological and pathological processes [18, 19].

Previous studies identified miRNAs as critical regulators of cardiac remodeling, myocardial function, cardiac metabolism and cardiovascular disorders [20-23]. Recently, studies have provided evidence of the role of miRNAs in obesity-related cardiovascular dysfunction [24-26]. Previously, we demonstrated that high-fat diet-induced cardiac hypertrophy in male mice is accompanied by alteration of miRNA expression profiles and that miRNA-22 was among the most upregulated miRNAs [27]. miRNA-22 is a key regulator of cardiac remodeling and myocardial function in response to stress [28, 29]. Recently, we reported that although deletion of miRNA-22 did not affect high-fat diet-induced cardiac hypertrophy in male mice, it was sufficient to attenuate adiposity and prevent dyslipidemia [30].

Given that miRNA-22 targets the estrogen receptor α (ERα) [31] and exerts a sex-specific regulation of body weight gain by repressing ERα [32], here we investigated the impact of miRNA-22 deletion in obesity-induced cardiovascular and metabolic disorders in female mice.

Materials and Methods

Animals and experimental diets

The animal experiments were performed after approval by the ethical committee for experimental animal care at the Institute of Biomedical Sciences of the University of Sao Paulo (#66/2016/CEUA). The animal studies were performed in the Animal Facility of the Department of Anatomy at the Institute of Biomedical Sciences of the University of Sao Paulo (Sao Paulo, Brazil). In this study, we used global miRNA-22 knockout (miR-22 KO) and wild type (WT) female mice obtained from heterozygous breeding, as described previously [28]. Genotyping was performed at 2 weeks of age using PCR. Five-week-old WT and miR-22 KO females were randomly fed a control diet (chow diet, 70% kcal carbohydrate, 10% fat kcal, 20% protein kcal, 3.87 kcal/g) or an obesogenic diet containing a high-fat diet (HF diet, 20% carbohydrate kcal, 60% fat kcal, 20% protein kcal, 5.24 kcal/g) and sweetened condensed milk (55% simple sugar, 13% fat, 7% protein, 3.25 kcal/g, Nestlé) supplemented with a mineral and vitamin mix for 16 weeks. The obesogenic diet was based in a previous study [33] with minor modifications. Mice were housed in a 12-hour light-dark cycle at 22±1°C and had *ad libitum* access to water and food. Body weight gain was examined weekly for 16 weeks. Euthanasia was performed using a carbon dioxide chamber. After euthanasia, heart, retroperitoneal adipose tissue (RAT), perigonadal adipose tissue (PAT), subcutaneous adipose tissue (SAT) and gastrocnemius muscle were collected, weighted and stored at -80°C.

Magnetic resonance imaging

After 16 weeks of dietary feeding, body composition was evaluated using nuclear magnetic resonance imaging (Bruker's minispec LF50 Body Composition Analyzer).

Glucose tolerance test and insulin tolerance test

Following 15 weeks of dietary feeding, mice were fasted overnight. Intraperitoneal glucose tolerance tests (iGTT) were performed by assessing blood glucose levels before and after an intraperitoneal injection of glucose (2 g/kg body weight) using a glucometer (Accu-Chek® Active, Roche Diagnostics) for 2 hours and the area under the curve (AUC) was calculated. After 72 hours, insulin tolerance tests (ITT) were performed in mice fasted for 6 hours. Mice received an insulin injection (0.5 U insulin/kg BW) and the glucose levels were assessed before and after insulin administration for 60 minutes. Blood glucose disappearance rate (KITT) was calculated to evaluate insulin sensitivity.

Echocardiography

Echocardiographic measurements were performed to characterize cardiac morphology and function after 16 weeks of dietary feeding, as previously described [34]. Mice were anesthetized with isoflurane, and images were obtained using a Vevo 2100 system (Visual Sonics) with a 13-24-MHz MicroScan transducer (model MS-550D). The following measurements were obtained: End-systolic interventricular septum (IVSs), End-diastolic interventricular septum (IVSd), LV end-systolic posterior wall thickness (LVPWs), LV end-diastolic posterior wall thickness (LVPWd), fractional shortening (FS), ejection fraction (EF), isovolumetric relaxation time (IVRT), and isovolumetric contraction time (IVCT); transmitral valve flow velocity in early diastole (E) and atrial systole (A) waves.

Hemodynamic parameters

Systolic blood pressure (SBP) and heart rate (HR) were measured after 15 weeks of dietary feeding using tail-cuff plethysmography (BP-2000 Blood Pressure Analysis System™ of Visitech Systems©). To avoid stress-induced variations, mice were adapted to the device for one week before measurements. Ten measurements of SBP and HR were collected per mouse.

Biochemical measurements

Plasma levels of triglycerides and total cholesterol were quantified using colorimetric assays (Wako Diagnostics).

Histology

Heart and PAT samples were fixed in 4% paraformaldehyde overnight. After dehydration, samples were embedded in paraffin and sectioned into 5 μm-thick slices. Heart sections were stained with wheat germ agglutinin for quantification of cardiomyocyte area at the papillary muscle level (n=50 cardiomyocytes per heart containing visible cell limits, n=3-4 hearts per group). In addition, Picrosirius Red staining was used to evaluate collagen deposition in the cardiac tissue (n=3-4 hearts per group). Perigonadal adipose tissue sections were stained with hematoxylin and eosin for quantification of adipocyte size (n= 40 adipocytes, n=3-4 mice per group). Images were obtained using a light microscope (Nikon® and software Nis-Elements) and examined using ImageJ software.

Western Blotting

Protein from gastrocnemius muscle and PAT samples was isolated using RIPA buffer (Millipore) with protease and phosphatase inhibitors. Protein concentrations were determined using a BCA protein assay kit (Thermo Fisher). Forty μg of protein was resolved by electrophoresis in an SDS-polyacrylamide gel and transferred onto nitrocellulose membranes (Bio-Rad). The membrane was incubated at 4°C with primary antibodies for ALDH2 (1:1000, #48837 - Santa Cruz Biotechnology), AMPK (1:1000, #2532 - Cell Signaling Technologies), p-AMPKα [Thr172] (1:1000, #2535 - Cell Signaling Technologies), ACC (1:1000, #3676 - Cell Signaling Technologies), p-ACC (1:1000, #11818 - Cell Signaling Technologies), α-tubulin (1:1000, #5286 - Santa Cruz Biotechnology) and UCP1 (1:1000, #14670 - Cell Signaling Technology) for 1 hour at room temperature. The membranes were washed and incubated with a horseradish peroxidase-conjugated secondary antibody (1:10000, Amersham Biosciences). Then, the membranes were washed and bands were visualized by chemiluminescence using a ClarityTM Western ECL Substrate detection system (Bio-Rad) following the manufacturer's instructions. The protein levels were measured by densitometry (ImageJ software). Ponceau staining of the membrane was used to normalize the ALDH2 protein levels. The results are expressed as relative levels in relation to control.

Muscle enzymatic activity

Activity of key enzymes of energetic metabolism - hexokinase (HK), fructose 6 phosphate dehydrogenase (PFK), pyruvate kinase (PK), citrate synthase (CS), creatine kinase (CK), lactate dehydrogenase (LDH), carnitine palmitoyltransferase (CPT-1), and β-hydroxyacyl CoA dehydrogenase (BHADH) - were analyzed in the gastrocnemius muscle of the mice. Samples were homogenized 1:10 (wt/vol) in 50 mM Tris-HCl, 1 mM EDTA, and protease inhibitor cocktail (pH 7.4). The lysate was centrifuged at 12000 rpm for 10min at 4°C and the supernatant collected. All enzyme activities were determined at 25°C using a Spectra Max 250 microplate spectrophotometer (Molecular Devices, Sunnyvale, CA). The assay buffer without sample was used as blank. The activity of these enzymes was determined as previously described [35-41].

Quantitative RT-PCR (qPCR)

Total RNA was isolated from heart samples using Trizol (Life Technologies). One μg of RNA samples was reverse-transcribed to cDNA using SuperScript II RNase H Reverse Transcriptase (Invitrogen). PCR was performed using SYBR Green PCR Master Mix (Applied Biosystems), according to the manufacturer's protocol in thermocycler (Corbett Research). The relative gene expression levels were calculated using the formula (2-ΔΔCt).

The primers used were, for 18S: 5'-GCCACTTGTCCCTCTAAGAAGTTG-3' and 5'-GTGCATGGCCGTTCT-TAGTTG-3'; for alpha-myosin heavy chain (Myh6): 5'-TGACGTCACCTCCAACATGG-3' and 5'-CCAACTCCCC-GTTCTCTGTC-3'; for beta-myosin heavy chain (Myh7): 5'-ATTGGTGCCAAGGGCCTGAAT-3' and 5'-GCTTC-CACCTAAAGGGCTGTTG-3'; alpha skeletal muscle actin (Acta-1): 5′-GCTCGGTGAGGATTTTCATCA-3′ and 5′-CCTGCCACACGCCATCAT-3'; collagen 1 (Col1a): 5′-TTCTCCTGGCAAAGACGGAC-3′ and 5′-CGGC-CACCATCTTGAGACTT-3'; collagen 3 (Col3a): 5′-ACGTAAGCACTGGTGGACAG-3′ and 5′-CAGGAGGGC-CATAGCTGAAC-3'; epidermal growth factor receptor (Egfr) 5′-CACGCCAACTGTACCTATGGATGT-3′ and 5′-GGCCCAGAGGATTTGGAAGAA-3'; ras homolog family member A (Rhoa): 5′-CCTTTTGCATTGAACGTG-GATT-3′ and 5′-ACTTCTCAGATGCAAGGACAA-3'; transforming growth factor beta receptor 1 (Tgfbr1): 5′-CCTCGAGACAGGCCATTTGT'-3' and 5′-CAGCTGACTGCTTTTCTGTAGTT-3'; calmodulin 1 (Calm1): 5′-CGAGTGTTTGACAAGGATGGG-3′ and 5′-ACTTGTCCGTCGCCATCAAT-3'; heat shock protein 90 alpha family class B member 1 (Hsp90ab1): 5′-GCTCCTTCGCTATCACACCT-3′ and 5′-TTGCTCTTTGCTCTCACCAGT-3';

peptidylprolyl isomerase A (Ppia): 5′-AGGATTCATGTGCCAGGGTG′-3′ and 5′-GCCATCCAGCCATTCAGTCT-3′; thyroid hormone receptor alpha (Thra): 5′-GGCGTTTGAGCACTACGTCA-3′ and 5′-TGCGGACCCTGAA-CAACAT-3′; peroxisome proliferative activated receptor gamma, coactivator 1 alpha (Pgc1a): 5′-GGGT-TATCTTGGTTGGCTTTATG-3′ and 5′-AAGTGTGGAACTCTCTGGAACTG-3′; yes1 associated transcriptional regulator (Yap1): 5′-GTGGAGGCAGTTCCAACCAG-3′ and 5′-ATTCCGTATTGCCTGCCGAA-3′. 18S was used as endogenous control since its expression was not affected either by obesogenic diet or by deletion of miRNA-22. Total RNA isolated from heart samples was reverse transcribed using specific primers of TaqMan® MicroRNA Assays (Applied Biosystems) for miRNA-22-3p, miRNA-22-5p, and snoRNA 234. Samples of cDNA were amplified by qPCR using TaqMan® Universal PCR Master Mix (Applied Biosystems) and primers for detection. The relative expression levels of miRNA-22-3p and miRNA-22-5p were normalized to snoRNA 234 levels, which were unchanged among groups.

RNA-sequencing data

Total RNA isolated from the hearts using Trizol (Invitrogen) was used for RNA-seq analysis (two biological replicates for group). Sequencing libraries were obtained using TruSeq® Stranded mRNA Library Prep (Illumina®) following the manufacturer's instructions. The library preparations were sequenced on an Illumina Nextseq platform according to the manufacturer´s instructions using NextSeq 500 High Output Kit v2.5, 1x75cycles.

Gene expression quantification and differential gene expression analyses

Gene expression levels were quantified by Kallisto [42] using mouse reference transcriptome from GENCODE (version M24; https://www.gencodegenes.org/). The conversion from transcript expression to gene expression was performed by R package txImport [43] and genes with expression higher than 1 count in any sample were kept. The gene expression matrix generated was used as the input to perform differential expression analysis using DESeq2 [44]. All protein-coding genes presenting a False Discovery Rate (FDR) < 0.05 and logarithm Fold Change (log2FC) < -1 or > 1 in the comparisons were defined as differentially expressed and used for the downstream analyses. For graphical representations of these genes, we used the R packages pheatmap (Version 1.0.12) to make the heatmaps and ggplot2 (Version 3.3.0) [45].

Functional Enrichment Analysis

The enriched biological pathways for differentially expressed genes were assessed using Gsea web server [46], which included the Gene Ontology (GO) biological processes. A value of adjusted p-value < 0.05 was defined as a significant cut-off for enrichment. The Venn Diagram analysis was performed using Venny v.2.1 (https://bioinfogp.cnb.csic.es/tools/venny/).

Statistical analyses

Results are expressed as mean ± SEM. Statistical analysis was performed using two-way ANOVA followed by Tukey or Bonferroni post-hoc tests for multiple comparisons, as appropriate, using Prism GraphPad®. Statistical significance was set to a $p \leq 0.05$.

Results

Deletion of miRNA-22 attenuates obesogenic diet-induced body weight gain and adiposity in female mice

To investigate the effect of miRNA-22 deletion in diet-induced cardiometabolic disorders in females, we used systemic miR-22 KO and WT female mice fed an obesogenic or chow diet. As expected, an obesogenic diet increased body weight gain in WT females (Fig. 1A). However, miR-22 KO females fed an obesogenic diet exhibited lower body weight gain compared to WT mice fed the same diet, suggesting that loss of miRNA-22 attenuates diet-induced obesity in females. No difference was observed in body weight gain between miR-22 KO and WT females fed a chow diet.

Next, we assessed the body composition using magnetic resonance imaging. Both WT and miR-22 KO females fed an obesogenic diet exhibited increased body fat percentage

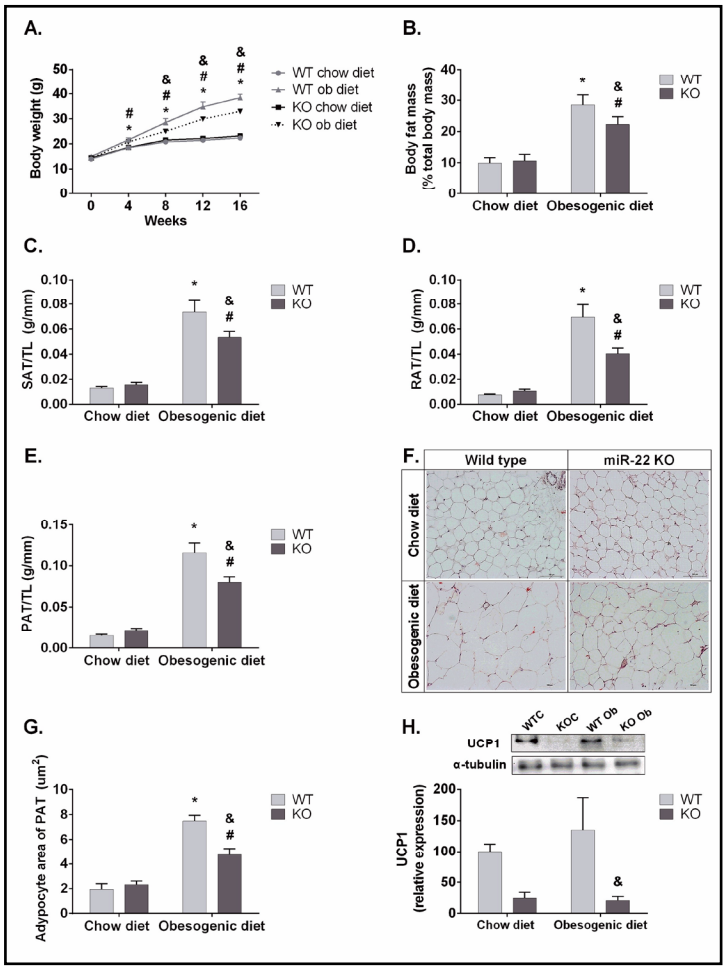

Fig. 1. Deletion of miRNA-22 attenuates diet-induced obesity in female mice. (A) Analysis of body weight gain in WT and miR-22 KO females fed a chow diet or obesogenic diet for 16 weeks (n=7-14). (B) Percentage of body fat mass evaluated by nuclear magnetic resonance imaging (n=7-12). (C) Subcutaneous adipose tissue weight normalized by tibia length (SAT/TL); (D) Retroperitoneal adipose tissue (RAT/TL); (E) perigonadal adipose tissue (PAT/TL) (n=7-12). (F) Analysis of adipocyte area in PAT using hematoxylin eosin staining and (G) quantification of adipocyte area evaluated by ImageJ; bars 100µm (n=3-4). (H) UCP1 levels in PAT evaluated by western blot (n=3-4). * WT obesogenic diet vs WT chow diet ($p<0.05$); # KO obesogenic diet vs KO chow diet ($p<0.05$); & KO obesogenic diet vs WT obesogenic diet ($p<0.05$).

compared with their respective controls fed a chow diet (Fig. 1B). However, body fat percentage in miR-22 KO females fed an obesogenic diet was lower compared with WT fed the same diet. More specially, we found that an obesogenic diet increased the weight of subcutaneous (SAT), retroperitoneal (RAT), and perigonadal (PAT) adipose tissues both in WT and miR-22 KO females (Fig.1C-1E); however, this increase was mitigated in obesogenic diet-fed miR-22 KO females compared to WT females fed the same diet.

Histological analysis of PAT using hematoxylin and eosin staining revealed that obesogenic diet increased adipocyte size in WT females (Fig. 1F-G). In contrast, loss of miRNA-22 attenuated obesogenic diet-induced increases in adipocyte size. Together, these data suggest that deletion of miRNA-22 attenuates obesogenic diet-induced adiposity in females.

Considering that WAT browning has been shown to reduce adiposity, we next verified whether deletion of miRNA-22 could induce browning of WAT. Western blot analysis revealed that miR-22 KO females fed both diets exhibited lower UCP1 levels in PAT compared to those in WT females (Fig. 1H). This finding suggests that WAT browning is not involved in reduction of adiposity found in miR-22 KO obese females.

miRNA-22 deficiency protects against obesogenic diet-induced dyslipidemia and insulin resistance in females

We therefore explored whether miRNA-22 might influence obesogenic diet-induced dyslipidemia in females. The obesogenic diet increased triglycerides and total cholesterol levels in WT females (Fig. 2A-B). However, miR-22 KO females did not exhibit increases

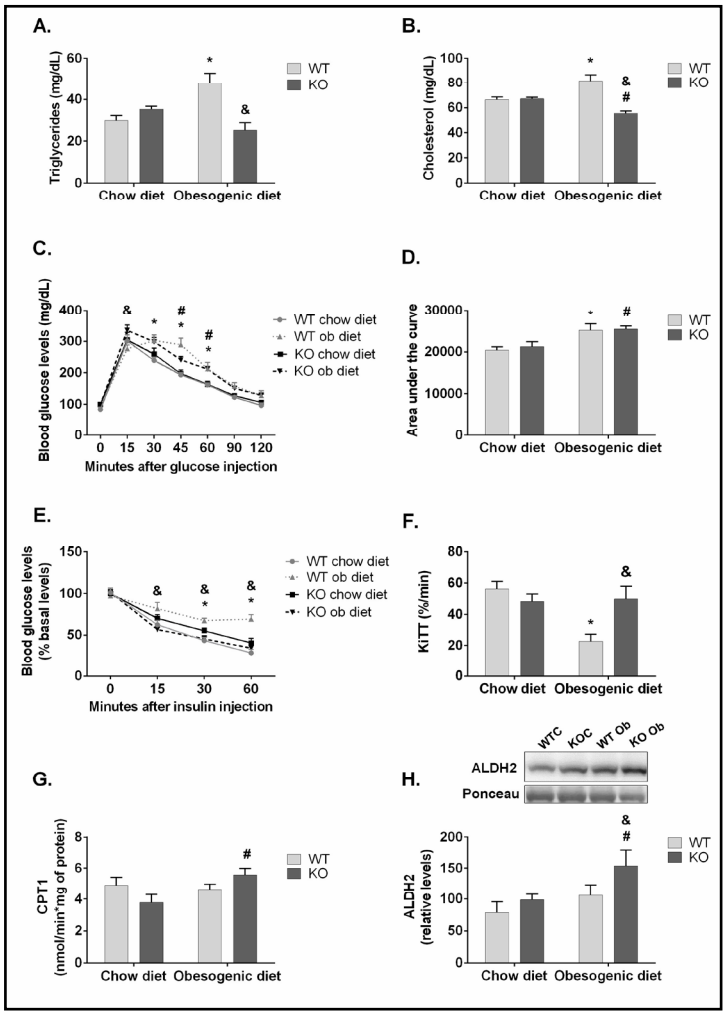

Fig. 2. Loss of miRNA-22 prevents obesogenic diet-induced dyslipidemia and insulin resistance in females. (A) Plasma triglycerides levels and (B) total cholesterol levels in WT and miR-22 KO females (n=5-8). (C) Analysis of intraperitoneal glucose tolerance test (iGTT) and (D) area under the curve (AUC) (n=7-12). (E) Insulin tolerance test (ITT) and (F) blood glucose disappearance rate (KITT) (n=4-9). (G) Activity of CPT1 in gastrocnemius muscle (n=5-9). (H) Relative protein levels of ALDH2 in gastrocnemius muscle of the mice (n=4-9). * WT obesogenic diet vs WT chow diet ($p<0.05$); # KO obesogenic diet vs KO chow diet ($p<0.05$); & KO obesogenic diet vs WT obesogenic diet ($p<0.05$).

in triglycerides and total cholesterol levels in response to an obesogenic diet, suggesting that deletion of miRNA-22 prevented obesogenic diet-induced dyslipidemia in females. No significant changes were detected in triglycerides and total cholesterol levels between WT and miR-22 KO females fed a chow diet.

Next, we examined whether miRNA-22 could influence glucose intolerance and insulin resistance in response to an obesogenic diet in females. Both WT and miR-22 KO females fed an obesogenic diet developed glucose intolerance, as evidenced by iGTT and area under the curve (Fig. 2C-D). Loss of miRNA-22 did not affect obesogenic diet-induced glucose intolerance.

WT females fed an obesogenic diet developed insulin resistance, as assessed by reduced blood glucose disappearance rate (KITT) (Fig. 2E-F). Nonetheless, miR-22 KO females fed an obesogenic diet exhibited KITT similar to that observed in WT females fed a chow diet, suggesting that deletion of miRNA-22 protected females against obesogenic diet-induced insulin resistance.

Given that skeletal muscle plays a key role in insulin-mediated glucose uptake [47], we verified whether miRNA-22 might affect skeletal muscle metabolism. Body composition analysis using magnetic resonance imaging revealed that an obesogenic diet reduced lean mass percentage in WT females (Table 1). However, the lean mass percentage was increased in obesogenic diet-fed miR-22 KO females compared with WT females fed the same diet. Western blot analysis revealed that the phosphorylation levels of AMPK (pAMPK), which increases glucose uptake and fatty acid oxidation, were reduced in the skeletal muscle of

WT and miR-22 KO females fed an obesogenic diet compared to their respective controls fed a chow diet (Supplementary Fig. 1A – for all supplementary material see www.cellphysiolbiochem.com). In addition, phosphorylation levels of ACC (pACC), which increases fatty acid oxidation, were similar among the groups (Supplementary Fig. 1B).

Next, we evaluated the effect of miRNA-22 and obesogenic diet in the activity of enzymes involved in glucose metabolism in gastrocnemius muscle of females. Hexokinase (HK), phosphofrutokinase (PFK) and pyruvate kinase (PK) activities were unchanged among groups (Table 1). Similarly, the activity of creatine kinase (CK), which plays an important role in energetic metabolism, was unaffected by miRNA-22 deletion or an obesogenic diet (Table 1). In addition, the activity of citrate synthase (CS), which is a key enzyme of oxidative metabolism, was similar among groups (Table 1).

We therefore sought to determine whether deletion of miRNA-22 might change fatty acid oxidation in the skeletal muscle of females. The activity of β-hydroxyacyl-CoA dehydrogenase (BHADH), which is an enzyme involved in fatty acid oxidation, was increased in miR-22 KO females fed a chow diet compared to WT females fed the same diet (Table 1). Moreover, carnitine palmitoyltransferase I (CPT1) activity, also involved in fatty acid oxidation, was enhanced in the gastrocnemius muscle of miR-22 KO females fed an obesogenic diet compared to their respective controls (Fig. 2G).

We next verified whether miR-22 KO females exhibited changes in mitochondrial content in skeletal muscle. Western blot analysis demonstrated that aldehyde dehydrogenase (ALDH2), an enzyme located at the mitochondrial matrix, was increased in the skeletal muscle of miR-22 KO females fed an obesogenic diet (Fig. 2H). Collectively, these results suggest that deletion of miRNA-22 increases mitochondrial fatty acid β-oxidation in the skeletal muscle of obese females.

Deletion of miRNA-22 induces cardiac hypertrophy in females but does not affect obesogenic diet-induced cardiac hypertrophy

We reported that miRNA-22 expression is increased in high-fat diet-induced cardiac hypertrophy in male mice [30]. We therefore examined whether the obesogenic diet could affect the expression levels of miRNA-22 in the heart of females. Analysis of qPCR revealed that miRNA-22-3p levels were increased in obesogenic diet-induced cardiac hypertrophy in females without modulation of miRNA-22-5p levels (Fig. 3A).

Next, we verified whether deletion of miRNA-22 could affect the obesogenic diet-induced cardiac hypertrophy in females. Obesogenic diet induced cardiac hypertrophy in WT females, as evaluated by the increased heart weight to tibia length ratio (HW/TL) (Fig. 3B). Interestingly, the HW/TL was increased in miR-22 KO females fed a chow diet. These results indicate that deletion of miRNA-22 promotes cardiac hypertrophy in female mice, which was unchanged by an obesogenic diet.

We therefore performed histological and molecular analysis to characterize the impact of miRNA-22 loss and an obesogenic diet in cardiac remodeling of females. Heart transverse sections stained with wheat germ agglutinin (WGA) revealed that obesogenic diet induced cardiomyocyte hypertrophy in WT females, as evidenced by increased cardiomyocyte area (Fig. 3C-D). Similarly, cardiomyocyte area was increased in miR-22 KO females fed both diets compared to chow diet-fed WT females. Analysis of qPCR revealed that the expression levels of cardiac

Table 1. Skeletal muscle features. * WT obesogenic diet vs WT chow diet ($p<0.05$); # KO obesogenic diet vs KO chow diet ($p<0.05$); & KO obesogenic diet vs WT obesogenic diet ($p<0.05$); § KO chow diet vs WT chow diet ($p<0.05$)

Parameter	Chow diet		Obesogenic diet	
	Wild type (n=8-11)	miR-22 KO (n=4-12)	Wild type (n=6-8)	miR-22 KO (n=8-10)
Lean Mass (%)	72.70 ± 0.50	72.78 ± 0.57	54.99 ± 0.96 *	61.20 ± 0.79 #&
PK (umol/min*mg)	413.49 ± 18.88	410.74 ± 60.10	455.26 ± 23.90	406.51 ± 19.70
HK (umol/min*mg)	3.67 ± 1.25	5.58 ± 1.38	5.78 ± 0.44	8.13 ± 1.82
PFK (umol/min*mg)	85.13 ± 5.57	77.92 ± 6.77	74.67 ± 2.77	72.26 ± 3.94
CK (umol/min*mg)	50.69 ± 1.96	46.46 ± 4.58	45.51 ± 1.99	51.06 ± 3.96
CS (umol/min*mg)	107.81 ± 4.10	112.52 ± 14.03	110.86 ± 10.30	120.96 ± 3.66
BHADH (umol/min*mg)	24.21 ± 4.91	42.54 ± 9.97§	39.02 ± 3.34	47.89 ± 3.03

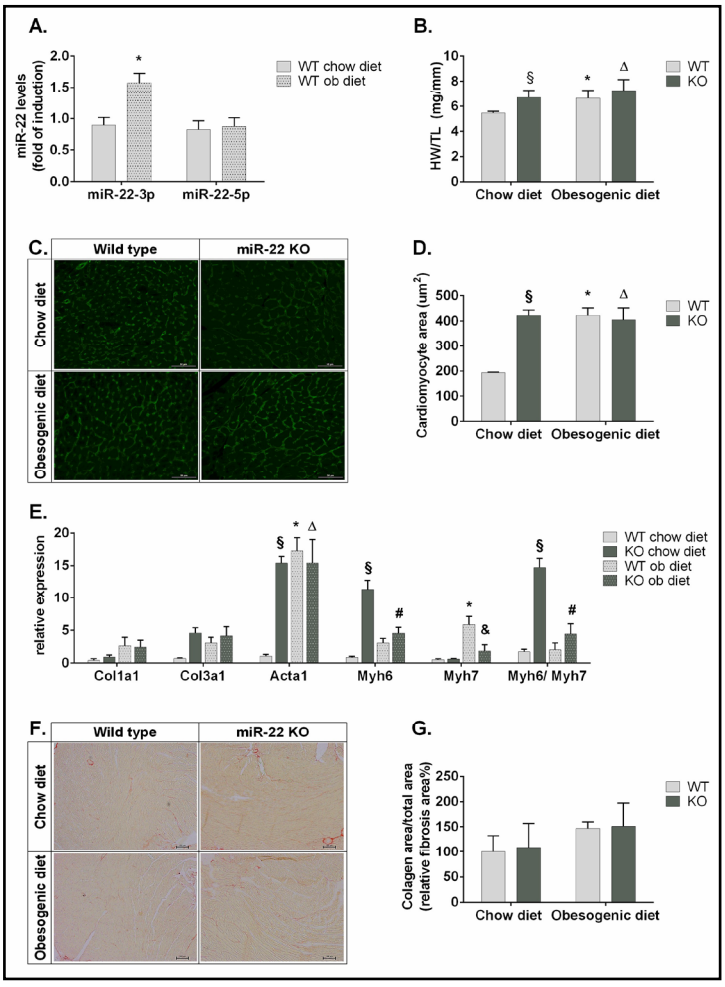

Fig. 3. Deletion of miRNA-22 induces cardiac hypertrophy in female mice. (A) miR-22 expression levels in hearts of WT female mice fed a chow diet or obesogenic diet analyzed by qPCR (n=5-9). (B) Heart weight normalized by tibia length (HW/TL) (n=7-11). (C) Analysis of cardiomyocyte area of heart transverse sections using wheat germ agglutinin staining and (D) quantification of cardiomyocyte area evaluated by ImageJ; bars 50µm (n=3-4). (E) Analysis of gene expression of hypertrophic markers and collagen evaluated by qPCR in the heart of the mice (n=3-5). (F) Analysis of fibrotic area of heart transverse sections using Picrosirius Red staining and (G) quantification of fibrosis area evaluated by ImageJ; bars 100µm (n=2-4). * WT obesogenic diet vs WT chow diet (p<0.05); # KO obesogenic diet vs KO chow diet (p<0.05); & KO obesogenic diet vs WT obesogenic diet (p<0.05); § KO chow diet vs WT chow diet (p<0.05); Δ KO obesogenic diet vs WT chow diet (p<0.05).

hypertrophy markers, such as *Myh7* and *Acta-1*, were increased in the hearts of WT obese females (Fig. 3E). In addition, miR-22 KO females fed both diets exhibited increased expression levels of *Myh6* and *Acta-1* compared to those found in WT females fed a chow diet. Interestingly, miR-22 KO obese females displayed lower expression levels of *Myh6* and the *Myh6*/*Myh7* ratio compared to their respective controls fed a chow diet (Fig. 3E). Together, these findings suggest that deletion of miRNA-22 *per se* induces cardiac hypertrophy in females and miRNA-22 loss does not affect obesogenic diet-induced cardiac hypertrophy in female mice.

Table 2. Cardiovascular and echocardiographic parameters. * WT obesogenic diet vs WT chow diet (p<0.05); # KO obesogenic diet vs KO chow diet (p<0.05); § KO chow diet vs WT chow diet (p<0.05)

Parameter	Chow diet		Obesogenic diet	
	Wild type (n=4-7)	miR-22 KO (n=5-8)	Wild type (n=4-7)	miR-22 KO (n=6-11)
HR (bpm)	656 ± 14	685 ± 13	675 ± 8	650 ± 9
SBP (mmHg)	104.11 ± 0.95	103.72 ± 5.13	99.65 ± 3.87	94.79 ± 4.31
IVSd (mm)	0.60 ± 0.03	0.64 ± 0.02 §	0.78 ± 0.01 *	0.77 ± 0.02 #
IVSs (mm)	0.84 ± 0.06	0.86 ± 0.02	1.07 ± 0.04 *	1.02 ± 0.05
LVPWd (mm)	0.61 ± 0.02	0.67 ± 0.03 §	0.77 ± 0.02 *	0.72 ± 0.03
LVPWs (mm)	0.84 ± 0.03	0.90 ± 0.04	1.09 ± 0.05 *	0.98 ± 0.05
LVIDd (mm)	4.01 ± 0.09	4.07± 0.07	3.92 ± 0.13	4.05 ± 0.08
LVIDs (mm)	2.98 ± 0.07	3.13 ± 0.09	2.75 ± 0.15	3.01 ± 0.11
EF (%)	50.90 ± 2.10	46.81 ± 2.65	57.48 ± 3.61	51.04 ± 2.55
FS (%)	25.65 ± 1.30	23.26 ± 1.59	30.05 ± 2.32	25.78 ± 1.52
IVRT (ms)	24.43 ± 2.52	22.50 ± 1.81	22.92 ± 1.03	20.05 ± 1.10
IVCT (ms)	26.88 ± 3.36	23.17 ± 2.46	20.56 ± 1.66	21.07 ± 1.18
E/A ratio	4.70 ± 0.88	3.02 ± 0.65	1.84 ± 0.15 *	2.28 ± 0.28

Next, we investigated the effect of miRNA-22 deletion and an obesogenic diet in myocardial fibrosis. No significant difference was noted in cardiac collagen content between WT and miR-22 KO females, as evaluated by histological analysis of heart transverse sections stained with Picrosirius Red (Fig. 3F-G). Consistent with this finding, the expression

levels of *Col1a1* and *Col3a1* were similar among groups (Fig. 3E). Taken together, these results suggest that deletion of miRNA-22 and an obesogenic diet induced cardiac hypertrophy in females without fibrosis.

We therefore evaluated whether miRNA-22 deletion and obesogenic diet could affect hemodynamic and echocardiographic parameters in females. Heart rate (HR) and systolic blood pressure (SBP) were measured using tail cuff plethysmography. WT and miR-22 KO females fed a chow diet or an obesogenic diet had similar SBP and HR values (Table 2). Echocardiographic analysis revealed that the interventricular septum thickness in diastole (IVSd) was increased in miR-22 KO females fed both diets, as well as in WT females fed an obesogenic diet (Table 2). In addition, left ventricular posterior wall thickness in diastole (LVPWd) was enhanced in miR-22 KO females fed a chow diet and in WT females fed an obesogenic diet (Table 2). No changes were detected in left ventricular internal dimension in diastole (LVIDd) and systole (LVIDs) between WT and miR-22 KO females (Table 2). Ejection fraction (EF) and fractional shortening (FS) were unchanged by deletion of miRNA-22 or an obesogenic diet (Table 2). However, the E/A ratio, a marker of LV function, was reduced in WT females fed an obesogenic diet (Table 2).

Deletion of miRNA-22 and an obesogenic diet change mRNA expression profiles in female hearts

To better understand the molecular mechanisms involved in cardiac hypertrophy induced by deletion of miRNA-22 and an obesogenic diet in females, we performed RNA sequencing (RNA-seq) to examine alterations in gene expression. Transcriptional profiles revealed that hearts from miR-22 KO females fed a control diet exhibited up- (2,977 genes) and downregulation (627 genes) of genes compared to WT females fed the same diet (Fig. 4A). Moreover, WT obese females demonstrated both up- (1,888 genes) and downregulation (1,716 genes) of genes compared to WT females fed a chow diet (Fig. 4A). In addition, miR-22 KO obese females exhibited up- (184 genes) and downregulation (258 genes) of genes compared to WT mice fed a chow diet (Fig. 4A). Furthermore, the hearts from miR-22 KO obese females demonstrated up- (657 genes) and downregulation (2,947 genes) of genes in relation to miR-22 KO females fed a chow diet. Additionally, the transcriptome profiles of miR-22 KO obese females revealed up- (1,041 genes) and downregulation (2,563 genes) of genes compared to WT obese females (Fig. 4A). We performed hierarchical clustering analysis (heatmap) and showed that differentially expressed genes are clustered between the groups; in particular, we found that while there are differentially expressed genes between WT and miR-22 KO groups fed a normal chow diet, animals fed with an obesogenic diet (both WT and miR-22 KO) display greater differences than those fed a chow diet (Fig. 4B and Supplementary Fig. 2), suggesting that the obesogenic diet may impact gene expression more significantly than the loss of miRNA-22 in the hearts of females.

To identify biological pathways regulated either by deletion of miRNA-22 or obesogenic diet, we analyzed the differentially expressed genes using gene set enrichment analysis (GSEA) based on Gene Ontology biological processes. As expected, genes related to thyroid hormone response, heart growth, PI3K signaling and response to transforming growth factor beta were enriched in the hearts of miR-22 KO females fed a chow diet (Fig. 4C), consistent with prior reports [28, 48]. The enrichment analysis further revealed that genes associated with the regulation of the force of heart contraction, protein folding and fatty acid oxidation were enriched in response to an obesogenic diet in WT females (Fig. 4D). Moreover, miR-22 KO obese females displayed elevated expression of genes related to oxidative phosphorylation, response to interleukin 12 and protein folding compared to WT females fed a chow diet (Fig. 4E). Interestingly, we found that genes related to cell redox homeostasis, fatty acid beta oxidation and response to interleukin 12 were enriched in hearts from miR-22 KO obese females compared to their respective controls (Supplementary Fig. 3A). Furthermore, genes related to innate immune response, oxidative phosphorylation and interferon gamma were up-regulated in the hearts of miR-22 KO obese females compared to WT obese females (Supplementary Fig. 3B). The enrichment analysis of down-regulated genes is shown in Supplementary Fig. 4.

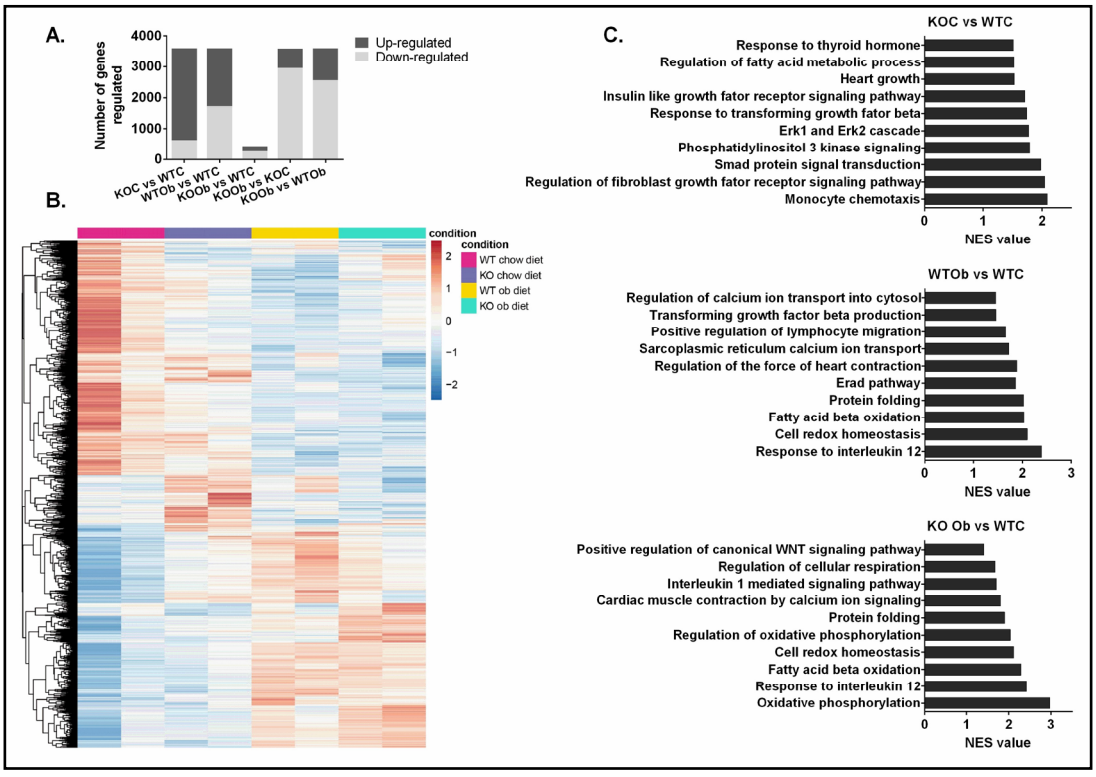

Fig. 4. Deletion of miRNA-22 and obesogenic diet change mRNA expression profiles in female hearts. (A) Number of genes regulated in the hearts of miR-22 KO and WT female mice fed a chow diet or obesogenic diet evaluated by RNA-seq (n=2). (B) Heatmap comparing the differential gene expression between WT obese, miR-22 KO fed a chow diet, and miR-22 obese females vs WT females fed a chow diet. The up-regulated genes are shown in red color and down-regulated genes in blue color (n=2). (C-E) Gene ontology (GO) enrichment analysis was performed using the Gsea, providing the up-regulated pathways enriched GO terms Biological Process (FDR≤0,25; p-value≤0.05; positive NES value).

To identify the overlap of differentially expressed genes between the groups with a hypertrophic phenotype, we generated a Venn diagram (Fig. 5A), which revealed five commonly altered genes, including *Acadl*, *Cav1*, *Hsp90ab1*, *Lonp2*, and *Rhoa*. Next, we performed qRT-PCR to examine the expression of a subset of genes related to cardiac hypertrophy. Consistent with RNA-seq data, qRT-PCR analysis revealed increased *Hsp90ab1* mRNA levels in groups with cardiac hypertrophy compared to WT females fed a chow diet (Fig. 5B). Although miR-22 KO females fed both diets and WT obese females had shown increased *Rhoa* expression in RNA-seq, qRT-PCR analysis revealed that *Rhoa* mRNA levels were significantly increased only in miR-22 KO females fed a chow diet (Fig. 5B). In agreement with RNA-seq data, *Calm1* mRNA levels were higher in miR-22 KO and WT obese females compared to their respective controls (Fig. 5B). RNA-seq analysis showed increased mRNA levels of *Ppia* both in WT and miR-22 KO obese females. However, qRT-PCR analysis revealed that *Ppia* mRNA levels were significantly increased in miR-22 KO obese females compared to WT obese females. In line with RNA-seq data, mRNA levels of *Yap1, Egfr,* and *Tgfbr1* were increased in miR-22 KO females fed a chow diet, as assessed by qRT-PCR (Fig. 5B). Finally, qRT-PCR analysis demonstrated that mRNA levels of *Thra* and *Pgc1a*, predict and validated targets of miRNA-22, respectively, were increased in miR-22 KO females fed both diets compared to WT females fed a chow diet (Fig. 5B).

In Fig. 6, we summarize the proposed mechanism for miRNA-22 action in obesity-induced disorders in females, as well as in cardiac hypertrophy.

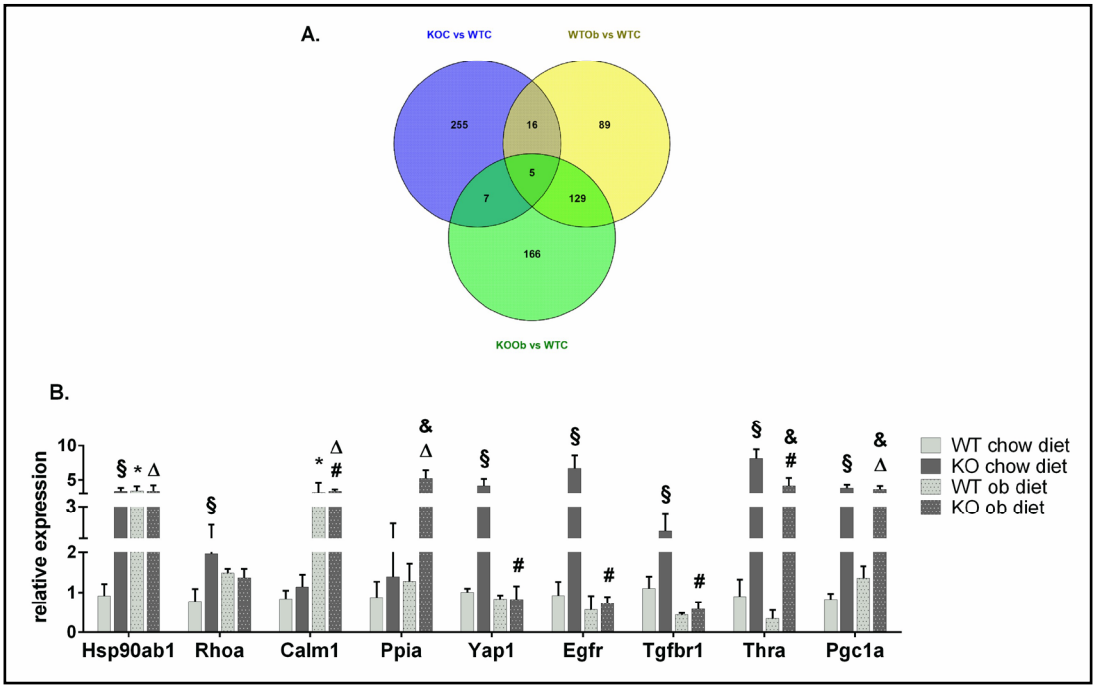

Fig. 5. Deletion of miRNA-22 and obesogenic diet change the expression of genes related to cardiac hypertrophy. A) Venn diagram showing the overlap of differentially expressed genes between the groups with hypertrophic phenotype. (B) Analysis of gene expression evaluated by qPCR in heart of the female mice (n=3-5). * WT obesogenic diet vs WT chow diet ($p<0.05$); # KO obesogenic diet vs KO chow diet ($p<0.05$); & KO obesogenic diet vs WT obesogenic diet ($p<0.05$); § KO chow diet vs WT chow diet ($p<0.05$); Δ KO obesogenic diet vs WT chow diet ($p<0.05$).

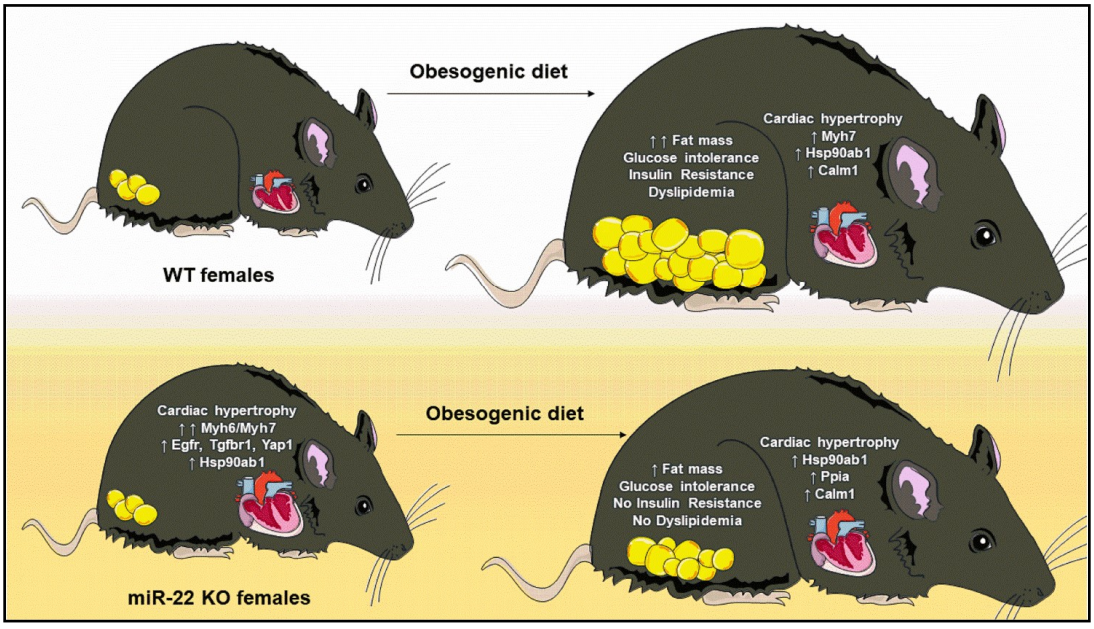

Fig. 6. Proposed mechanisms of action for miRNA-22 in females. Deletion of miRNA-22 attenuated body weight gain, adiposity, and prevented obesogenic diet-induced insulin resistance and dyslipidemia in females. Moreover, deletion of miRNA-22 and obesity induced cardiac hypertrophy in females. The increased mRNA levels of *Yap1*, *Egfr* and *Tgfbr1* in hearts induced by miRNA-22 deletion was prevented by obesogenic diet.

Discussion

In the present study, we found that loss of miRNA-22 attenuated obesogenic diet-induced body weight gain and adiposity in females. Also, we found that deletion of miRNA-22 protected females against insulin resistance and dyslipidemia in response to an obesogenic diet. In addition, our results suggest that deletion of miRNA-22 increases mitochondrial fatty acid β-oxidation in the skeletal muscle of obese females. Interestingly, deletion of miRNA-22 *per se* induced cardiac hypertrophy without affecting left ventricular function.

Here, we found that deletion of miRNA-22 did not affect body weight gain under a chow diet. This finding is consistent with a previous study [32], which demonstrated that inactivation of miRNA-22 in female mice did not change body weight gain in response to a chow diet. However, miR-22 KO females gained less body weight in response to an obesogenic diet, suggesting that deletion of miRNA-22 attenuates diet-induced body weight gain in females. This result differs from that observed by us in miR-22 KO male mice [30], suggesting that deletion of miRNA-22 may exert different effects in diet-induced body weight gain in a gender-dependent manner. Furthermore, loss of miRNA-22 attenuated obesogenic diet-induced adiposity and the increase in adipocyte size, indicating that miRNA-22 mediates, at least in part, the white adipose tissue expansion in response to an obesogenic diet in females. This finding is in agreement with our previous study in male mice [30], reinforcing the key role of miRNA-22 in the regulation of adiposity under hypercaloric diets in both males and females. Interestingly, we found that UCP1 levels were reduced in PAT of miR-22 KO females, suggesting that WAT browning probably is not involved in the reduction of adiposity observed in miR-22 KO obese females.

Consistent with our previous findings in males [30], deletion of miRNA-22 protected females against obesogenic diet-induced dyslipidemia. Together, these results highlight the importance of miRNA-22 as a key regulator in diet-induced dyslipidemia in both sexes. Although the exact mechanisms by which miRNA-22 deletion prevents diet-induced dyslipidemia are not completely known, we have demonstrated that loss of miRNA-22 prevented the increase in expression of proinflammatory markers and lipogenic genes in the liver of male mice in response to a high-fat diet [30]. However, further investigations are required to determine whether deletion of miRNA-22 exerts similar effects in response to an obesogenic diet in females.

In line with our previous research in males [30], miRNA-22 deletion did not affect obesogenic diet induced-glucose intolerance in females. Conversely, deletion of miRNA-22 prevented obesogenic diet-induced insulin resistance, suggesting that miRNA-22 plays a key role in insulin resistance associated with obesity in females.

Skeletal muscle is the major site for whole body insulin sensitivity and insulin resistance [47]. Nuclear magnetic resonance imaging revealed that miRNA-22 deletion attenuated the reduction in lean mass in response to an obesogenic diet in females. Both WT and miR-22 KO obese females exhibited reduced pAMPK levels in the skeletal muscle, while pACC levels were unchanged between groups. In addition, we found that both miRNA-22 loss and an obesogenic diet did not affect the activity of enzymes involved in glucose metabolism (HK, PFK and PK) and energy metabolism (CS) in the gastrocnemius muscle of females. However, the activity of CPT1, which plays a key role in fatty acid oxidation, was increased in the skeletal muscle of miR-22 KO females fed an obesogenic diet. A previous study showed that CPT1 activity is reduced in skeletal muscles of obese patients [49]. On the other hand, overexpression of CPT1 increased fatty acid oxidation and improved insulin sensitivity in high-fat diet-fed rats [50]. Therefore, it is possible that the improved insulin sensitivity in miR-22 KO obese females may be, in part, mediated by an increase in activity of CPT1 in the skeletal muscle. Inactivation of miRNA-22 increased the expression of genes involved in lipid metabolism in the skeletal muscle of male mice by repressing ERα [32]. Then, it would be interesting to investigate whether deletion of miRNA-22 could affect lipid metabolic processes in skeletal muscle in response to an obesogenic diet, and whether this effect could be influenced by sex. A recent study showed that miRNA-22 promotes skeletal muscle fiber

type conversion from fast-twitch to slow-twitch *in vitro* [51]. Patients with type 2 diabetes display reduced oxidative enzyme activity [52]. Given that slow oxidative fibers exhibit higher oxidative ability, changes in muscle fiber type caused by miRNA-22 deletion could affect energy metabolism and whole-body insulin sensitivity. However, this hypothesis needs to be further explored.

An obesogenic diet induced cardiac hypertrophy in WT females, as verified by increased HW/TL, cardiomyocyte area, and expression of hypertrophic markers. In addition, Picrosirius Red staining of heart transverse sections indicated that obesogenic diet-induced cardiac hypertrophy was not accompanied by myocardial fibrosis. This finding contrasts with previous reports, which revealed increased myocardial collagen deposition in obese females fed a western diet [53, 54]. The differences observed concerning the effect of an obesogenic diet on cardiac fibrosis in females highlights the key role of diet composition and time of treatment used to investigate obesity-induced cardiovascular disorders.

Obesity may induce hypertension [55], which can lead to cardiac hypertrophy. Several reports suggest that cardioprotection in women is reduced by type 2 diabetes mellitus and obesity [56]. Our results showed that cardiac hypertrophy in response to an obesogenic diet in females was not associated with hypertension. In line with this result, other studies have reported that diet-induced obesity both in females and males does not affect systolic blood pressure [27, 53, 57]. Our findings also revealed that obesogenic diet-induced cardiac hypertrophy in females was not associated with left ventricular dysfunction. Although we have not found left ventricular dysfunction in obese female and male mice [30], it is important to note that cardiac functional abnormalities have been demonstrated in diet-induced obesity in both sexes [53, 54, 58, 59].

In contrast with the results obtained in male mice [28-30], in this study we found that deletion of miRNA-22 in females *per se* induced cardiac hypertrophy. Furthermore, we found that cardiac hypertrophy in response to miRNA-22 deletion in females was independent of hypertension, myocardial fibrosis and left ventricular dysfunction. The reason for the discrepancy between miR-22 KO males and females is unclear. Therefore, it is possible that biological differences due to sexual dimorphism are responsible for the observed disparities. Furthermore, our results reinforce the importance of studying the impact of sex differences in the phenotype of knockout mouse models in both physiological and pathological conditions.

We detected that miRNA-22-3p expression levels were increased in obesogenic diet-induced cardiac hypertrophy in females. Although miR-22 KO females display cardiac hypertrophy, deletion of miRNA-22 did not exacerbate cardiac growth in response to an obesogenic diet. Interestingly, miR-22 KO obese females displayed a lower *Myh6*/*Myh7* ratio compared to their respective controls. Therefore, it would be interesting to examine whether deletion of miRNA-22 in females may affect myocardial remodeling and cardiac function after long-term exposition to obesogenic diet.

Some experimental interventions targeting adipose tissue have revealed beneficial effects for the cardiovascular system in preclinical and animal studies [8]. Given that body weight reduction attenuates cardiac hypertrophy associated with obesity [60, 61], it is possible that lower body weight and adiposity in miR-22 KO obese females may be contributing, at least, in part, to counteract cardiac growth in response to obesity.

To explore the potential mechanisms involved in cardiac hypertrophy in response to obesogenic diet and deletion of miRNA-22 in females, we examined the mRNA expression profile in hearts from WT and miR-22 KO females. Enrichment analysis revealed that genes associated with sarcoplasmic reticulum calcium transport, fatty acid oxidation, and response to interleukin 12 were enriched in WT obese females. In addition, *Hsp90ab1* and *Calm1* mRNA levels, which are involved in transforming growth factor beta production, protein folding and sarcoplasmic reticulum calcium ion transport, were higher in WT obese female hearts. Previous studies reported the role of these factors in diverse cardiac hypertrophy models, such as angiotensin II, isoproterenol, and left ventricle pressure overload by transverse aortic constriction [62-65]. However, further studies are needed to determine the impact of *Calm1* and *HSP90ab1* in obesity-induced cardiac hypertrophy in females.

In addition, genes related to the thyroid hormone response, heart growth, fibroblast growth factor receptor signaling, PI3K signaling, the Erk1 and Erk2 cascade, and the response to transforming growth factor beta were enriched in hearts from miR-22 KO females fed a chow diet. Moreover, *Hsp90ab1*, *Rhoa*, *Yap1*, *Egfr* and *Tgfbr1* mRNA levels were higher in miR-22 KO female hearts. Multiple studies have demonstrated the role of these factors in diverse models of cardiac hypertrophy [66-71]. Furthermore, mRNA levels of *Thra*, a predicted target of miRNA-22, and *Pgc1a*, a validated target of miRNA-22 [72], were enhanced in heart of miR-22 KO females. Nonetheless, future investigations are required to elucidate the contribution of these factors to obesity-associated cardiac hypertrophy.

RNA-seq data revealed that miR-22 KO obese females displayed elevated expression of genes related to oxidative phosphorylation, response to interleukin 12 and fatty acid beta oxidation. In addition, qPCR analysis showed increased mRNA levels of *Hsp90*, *Calm1*, *Thra,* and *Pgc1a* in hearts from miR-22 KO obese females. Although the role of *Ppia* has not been demonstrated in obesity-associated cardiac hypertrophy, previous studies showed its contribution in cardiovascular disorders, including Angiotensin II-induced myocardial hypertrophy and oxidative stress-induced cardiovascular dysfunction [73, 74]. Interestingly, miR-22 KO obese females exhibited reduced mRNA levels of *Yap1*, *Egfr* and *Tgfbr1* compared to those found in miR-22 KO females fed a chow diet, suggesting that inhibition of these genes may be preventing the exacerbated hypertrophic response induced by obesogenic diet in miR-22 KO females.

miRNA-22 exerts a sex-specific regulation of body weight gain by repressing estrogen receptor α [32]. However, our results revealed that miR-22 deletion attenuates diet-induced fat mass expansion and protects against dyslipidemia both in males [30] and females, suggesting that the beneficial role of miR-22 loss in obesity-related metabolic disorders is not sex-dependent. Moreover, we have not found estrogen signaling among the biological enriched pathways in the heart from miR-22 KO females fed a chow diet, suggesting that cardiac hypertrophy promoted by miR-22 deletion in females is not influenced by estrogen. For these reasons, in this study we have not evaluated the impact of estrogen (e.g., ovariectomy) in obesity-induced metabolic and cardiovascular alterations in females.

A series of studies performed in the last years identified that dysregulation of miRNAs has been linked to several disorders, including cardiovascular and metabolic diseases [21, 23, 75, 76]. Understanding the contribution of specific miRNAs to obesity-related cardiovascular and metabolic complications in both sexes is important given its potential for the development of innovative therapeutic strategies. Based on the results of our studies, it would be interesting to examine whether inhibition of miRNA-22 may be further used as a novel therapeutic approach for obesity-associated metabolic complications in both men and women.

Conclusion

Collectively, our findings support miRNA-22 as a potential target for diet-induced obesity, dyslipidemia and insulin resistance in females. In addition, our results highlight the importance of sexual dimorphism in the effects mediated by deletion of miRNA-22 in the heart.

Acknowledgements

RNA sequencing data have been deposited in the European Nucleotide Archive [ENA accession number: PRJEB39970].

Author Contributions

T.O.S. and G.P.D. designed the study, the protocols and wrote the manuscript. C.A.L., V.C.B., P.F.A., Y.W.L, and P.A.F.G performed RNA-seq and data analysis. V.M.L. and R.I.B.F performed the adipose tissue experiments. L.J and M.C.I performed echocardiography. G.M.M., S.V.F., M.A.C.R., J.C.B.F., and A.C.R. performed the skeletal muscle experiments. J.D.J. evaluated body composition. Z.P.H. generated miR-22 null mice. M.L.B.C. and D.Z.W. contributed with protocols and critical revision of the paper. G.P.D. is the guarantor of this study.

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Disclosure Statement

The authors declare that they have no conflict of interest.

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