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Grape polyphenols and exercise training have distinct molecular effects on cardiac hypertrophy in a model of obese insulin-resistant rats

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Abstract

Obesity and exercise lead to structural changes in heart such as cardiac hypertrophy. The underlying signaling pathways vary according to the source of the overload, be it physiological (exercise) or pathologic (obesity). The physiological pathway relies more on PI3K-Akt signaling while the pathologic pathway involves calcineurin-Nuclear factor of activated T-cells activation and fibrosis accumulation. Independently, exercise and polyphenols have demonstrated to prevent pathologic cardiac hypertrophy. Therefore, we investigated the molecular adaptations of the combination of exercise training and grape polyphenols supplementation (EXOPP) in obese high-fat fed rats on heart adaptation in comparison to exercise (EXO), polyphenols supplementation (PP) and high-fat fed rats (HF), alone. Exercised and PP rats presented a higher heart weight/body weight ratio compared to HF rats. EXO and EXOPP depicted an increase in cell-surface area, P-Akt/Akt, P-AMPK/AMPK ratios with a decreased fibrosis and calcineurin expression, illustrating an activation of the physiological pathway, but no additional benefit of the combination. In contrast, neither cell-surface area nor Akt signaling increased in PP rats; but markedly decreased fibrosis, calcineurin expression, systolic blood pressure, higher SERCA and P-Phospholamdan/Phospholamdan levels were observed. These data suggest that PP rats have a shift from pathologic toward physiological hypertrophy. Our study demonstrates that polyphenols supplementation has physical-activity-status-specific effects; it appears to be more protective in sedentary obese insulin-resistant rats than in the exercised ones. Exercise training improved metabolic and cardiac alterations without a synergistic effect of polyphenols supplementation. These data highlight a greater effect of exercise than polyphenols supplementation for the treatment of cardiac alterations in obese insulin-resistant rats.

Keywords: Obesity; Endurance; Heart; Insulin resistance; Metabolism.

1. Introduction

An unhealthy diet and a lack of physical activity contribute to increased blood pressure, cholesterol, overweight or obesity and, ultimately, some noncommunicable diseases [1–3]. Obesity, defined as excessive fat accumulation, is a major risk factor for insulin resistance (IR) and cardiovascular diseases (CVDs) [2]. In fact, IR is believed to be a primary contributor to metabolic and cardiovascular dysfunctions [4,5]. Decreased cardiac function in obesity has been associated with alteration of calcium (Ca²⁺) handling proteins [6,7]. Among these proteins, the sarcoplasmic reticulum Ca²⁺ ATPase (SERCA) protein has been shown to be the

main regulator of Ca²⁺ handling during the cardiac excitation-contraction-relaxation cycle. SERCA activity is directly modulated by the inhibitor phospholamban (PLN) on its dephosphorylated state by decreasing the affinity of SERCA for Ca²⁺. Thus, reduced phosphorylated PLN expression level has been linked to decreased cardiac contraction in obesity [6,8]. In addition to Ca²⁺ handling alterations, the cardiovascular modifications due to obesity comprise endothelial dysfunction, interstitial fibrosis, metabolic alterations, and cardiac hypertrophy [9–11]. Cardiac hypertrophy is an independent risk factor for myocardial infarction [12]. Cardiac hypertrophy, that is, an increase in heart weight normalized to body weight develops secondary to a repeated overload, decreases ventricular wall stress and allows normal cardiovascular function at rest [13]. Although the increase in heart weight primarily occurs through the hypertrophy of individual cardiomyocytes, the underlying molecular mechanisms vary according to the source of the overload, be it physiological or pathologic. For instance, pathologic cardiac hypertrophy relies on the calcium-calcineurin – Nuclear factor of activated T-cells (NFAT) pathway [13]. On the other hand, an adaptive and reversible physiological cardiac

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hypertrophy is observed with exercise training or pregnancy [14,15]. This physiological hypertrophy is closely interrelated to changes in metabolism [16] and mostly relies on the activation of the insulin-like growth factor-1 (IGF1)-phosphoinositide 3-kinase (PI3K)-Akt pathway [17]. Interestingly, Akt is a pivotal factor of cardiac hypertrophy as it plays a role in both the development of physiological and pathologic cardiac hypertrophy [18]. Specifically, Akt inhibits calcineurin and thus the pathologic hypertrophy pathway but also controls protein synthesis and degradation via the phosphorylation of mechanistic target of rapamycin (mTOR) and Forkhead box (FoxO) pathways [4,19,20]. Protein synthesis is further regulated by AMP-activated protein kinase (AMPK), which inhibits the mTOR anabolic pathway [21]. Interestingly, AMPK can be stimulated through physical activity and diet [22,23] in order to generate ATP. In fact, AMPK is centrally involved in the regulation of key enzymes of glucose and lipid oxidation, which provide intracellular ATP, by oxidative phosphorylation. Pathologic conditions such as obesity, type 2 diabetes, cardiac hypertrophy are associated with dramatic alterations in cardiac lipid and glucose metabolism [24].

Interventions reducing the impact of obesity rely on modifications of key behavioral risk factors such as unhealthy diet and lack of physical activity. Thus exercise training and diet are cornerstones of obesity treatments [22,23]. The important role of phytochemicals on human health has emerged during the last 15 years with an emphasis on polyphenols. These phytochemicals are secondary metabolites of plants and over 500 polyphenols compounds are present in plants foods representing dietary polyphenols [25]. Resveratrol, is a natural polyphenol present in many plant-based foods which has received great attention due to its ability to be an exercise- and calorie restriction mimetic [26,27]. Thus, resveratrol has been largely tested for the prevention of pathologic cardiac hypertrophy [28–31]. For instance, Chan et al. showed that resveratrol activated AMPK and prevented hypertrophy of cultured rat cardiomyocytes [21,32]. Interestingly, *in vitro* data show that resveratrol seems to prevent pathologic but not physiological cardiac hypertrophy [30]. However, several studies have demonstrated that total fruit intake, which presents the highest polyphenolic compounds and content sources, is better associated with improved cardiovascular health and lower deaths from CVDs than intake of polyphenolic compounds [33–35]. Furthermore, data from Lai et al. support that total fruit intake rather than intake of a specific type of fruit is protective against CVDs [36]. On the other hand, exercise ameliorates metabolic dysfunction and prevents chronic disease fighting against the disease of physical inactivity [37,38]. Although the combination of exercise and resveratrol has been effective in improving cardiac function in healthy rats [39], it remains to be seen whether these benefits persist in obese IR rat.

We recently investigated the combination of exercise training and a nutritional dose of a mixture of grape polyphenols, representative of a healthy diet, on obesity-related IR skeletal muscle and adipose tissue metabolic alterations [40,41]. Since IR is strongly correlated with the risk of CVDs, we investigated the molecular adaptations of the combination of exercise training and polyphenols supplementation in obese high-fat fed rats on heart metabolism.

2. Materials and methods

This study is part of a previously published protocol on the effect on a combination of grape polyphenols with exercise training [40,41].

2.1. Ethical approval

All animal experimentation procedures were conformed to the Directive 2010/63/EU that was adopted on the September 22, 2010 for the protection of

animals used for scientific purposes (agreement number: A34-172-38) and was approved by the Comité d'éthique en expérimentation animale Languedoc-Roussillon (CEEA-LR, C2EA-36) (protocol number: CEEA-LR-1062).

2.2. Animals

Forty 6-week-old male Sprague-Dawley rats, obtained from Janvier Laboratories (St Berthevin, France) were placed, after acclimatization, in the facility, and fed *ad libitum* with a high-fat diet (HFD, D12330 Research Diets, containing 25.5% carbohydrate, 58% fat, and 16.4% protein) for the 12 weeks of the experimental protocol. Rats were single-housed or double-housed in a temperature-controlled room and maintained with food and drink *ad libitum* in a 12:12 h light-dark cycle, lights on at 8:00 postmeridian. The training and *in vivo* tests were performed during rats' dark cycle exposition. Body mass was monitored every 2 days throughout the experimental period.

2.3. Experimental design

After 4 weeks of HFD, shown to induce skeletal muscle IR [42], the animals were divided into 4 groups ($n=10$ /group) while simultaneously continuing the HFD for an additional 8-week period. Group specificities were the following: (1) HFD alone (HF), (2) HFD and supplementation with grape polyphenols extract at 50 mg/kg/d per 50 mL of drinking water (PP), (3) HFD and exercise training, 1 h/d, 5 d/wk, consisting of running on a treadmill set at 32 m/min with a 10% slope (EXO), (4) HFD and PP supplementation combined with exercise training (EXOPP). The grape polyphenols extract used for supplementation (64.4% total polyphenols with 17.9% procyanidins, 4.3% anthocyanins, and 533 ppm resveratrol) had been previously used at this nutritional dose [43,44]. Animals were euthanized 48 hours after the last training session to avoid the acute effect of the last training session. After a 12-hour fast, animals were anesthetized with isoflurane and hemodynamic measurements were performed before being euthanized by exsanguination. Immediately after death, the heart was rapidly excised, weighed and frozen for western blot analysis and histologic analysis.

2.4. Blood analyses

2.4.1. Glucose tolerance test (GTT)

After a 12-hour fast, rats were injected intraperitoneally with a 30% glucose solution (2 g/kg body weight), as previously described [40,41]. Glucose level was determined at baseline and following glucose injection with a QuantiChrom Glucose Assay Kit (BioAssay Systems) on blood collected from the tail, insulin level was determined at baseline using Ultrasensitive Rat Insulin ELISA (Merckodia). Homeostasis model assessment of IR (HOMA-IR) index was calculated using the formula: $HOMA-IR = [\text{fasting insulin } (\mu\text{IU/mL}) \times \text{fasting glucose (mmol/mL)}] / 22.5$.

2.4.2. Determination of free fatty acid (FFA)

Plasma from 12-hour-fasted rats were collected from the carotid artery of anesthetized animals and centrifuged for 10 min at 2,000 g. FFA levels were determined using EnzyChrom Free Fatty Acid Assay Kit (EFFA, BioAssay System).

2.5. Hemodynamic measurements

Rats were anesthetized with isoflurane and a catheter (PE50; Merck-Bristol) was inserted into the right carotid artery, and systemic blood pressures, pulse pressure, and heart rate were recorded for 10 min.

2.6. Histologic analysis

Ventricular sections (10 μm) were stained with wheat germ agglutinin (WGA 1:250; Vector Labs) counterstained with 4',6-diamidino-2-phenylindole (DAPI 1:1000 from Sigma-Aldrich) and Masson's Trichrome for determination of cardiomyocytes cell-surface area (CSA) and fibrosis, respectively. In order to determine denaturated collagen (unfolded collagen), ventricular sections were incubated overnight with 20 μM of Collagen Hybridizing Peptide, Cy3 Conjugate (R-CHP from 3-Helix Inc) following manufacturer instructions and as previously described [45]. Since the 20 μM CHP solution needs to be heated at 80 °C for 5 min prior to incubation to hybridize with unfolded collagen strands, an aliquot of 20 μM CHP solution was not heated to act as a negative control [45]. Ventricular sections were observed with a Zeiss Axiolmager M1 microscope (Zeiss, Le Pecq, France) and TIFF images were captured with an Axiocam MRm Zeiss using Axiovision 4.7 (05-2008) software. Cardiomyocytes size was calculated using ImageJ software (National Institutes of Health, USA, available online at <http://rsbweb.nih.gov/ij/index.html>). Fibrosis and unfolded collagen strands (denaturated collagen) were quantified using GNU Image Manipulation Software (GIMP2.8). At least three fields per rat and three rats per group were averaged and used to perform quantification.

Table 1
Cardiovascular and metabolic characteristics determined in fasted condition

	HF	PP	EXO	EXOPP	Two-way ANOVA
Heart rate (bpm)*	322±12.2	306±14.1	276±14.1 ^{†‡}	330±12.2	Interaction $P=.$ 026
Systolic pressure (mm Hg)*	130±2.8	120±3.3	126.7±3.3	118.5±2.8	Supplementation $P<.$ 05
Diastolic pressure (mm Hg)*	91.2±3.5	93.3±4.0	95±4.0	89.2±3.5	$P>.$ 05
Body weight (g)	603.8±19.4	570.0±15.0	538.6±14.2	541.9±14.9	Exercise $P<.$ 05
Heart weight (g)	1.35±0.06	1.38±0.05	1.28±0.04	1.31±0.05	$P>.$ 05
FFA (μ M)	346.6±77.1	218.9±27.0	147.0±31.6	174.7±36.2	Exercise $P<.$ 05
Cholesterol (mg/dL)	62.8±3.6	63.1±3.3	57.7±7.2	69.7±4.3	$P>.$ 05
HOMA-IR	1.0±0.1	1.0±0.1 [†]	0.9±0.1 \$0.057	0.8±0.1 [‡]	Exercise $P<.$ 01

HF: High Fat Diet alone PP: High Fat Diet and supplementation with grape polyphenols extract EXO: High fat Diet and exercise training EXOPP: High Fat Diet and PP supplementation combined with exercise training ANOVA: two-way analysis of variance.

* Only 4 rats were used for this parameter.

[†] $P<.$ 05 versus EXOPP.

[‡] $P<.$ 05 versus HF.

2.7. Western blotting

Left ventricular tissues were homogenized in a Radioimmunoprecipitation Assay buffer supplemented with phosphatase and protease inhibitors (Sigma-Aldrich), followed by a 10-min centrifugation at 10,000 g. Immunoblots were then performed using the following primary antibodies: phospho-Akt (P-Akt, Ser473) (#4060, Cell Signaling Technology), total Akt (#9272, Cell Signaling Technology), phospho-AMPK (P-AMPK, Thr172) (#2531, Cell Signaling Technology), AMPK (#2532, Cell Signaling Technology), Pan-calcineurin A (#2614, Cell Signaling Technology), phospholamban (PLN) (ab85146, Abcam), phospho-PLN (P-PLN, Thr17) (A010-13AP, Badrilla), SERCA2 (ab2861, Abcam). GAPDH antibody (PA1-913, Fischer Scientific) was used as a loading control.

2.8. Enzymatic activities

Citrate synthase (CS) and hydroxyacylCoA dehydrogenase (HADH) activity. An aliquot (1 μ L for CS and 2.5 μ L for HADH) of left ventricular homogenate (see above paragraph) was used for enzyme activity measurements done in triplicate. CS activity was measured at 30 °C by following the formation of TNB generated from DTNB (5,5'-Dithiobis(2-nitrobenzoic acid)) (from Sigma-Aldrich) over 2 min at 412 nm, as previously described [43]. HADH activity was measured at 25 °C by following the formation of NAD generated from NADH (from Sigma-Aldrich) over 5 min at 340 nm, as previously described [43]. The variations in optical density during the kinetics are converted in micromoles per minute per g of tissue and expressed relative to HF value, which was set at 1.

2.9. Statistical analysis

Data are expressed as means \pm standard error of the mean (SEM). Comparisons between treatment groups Exercise versus Rest (EXO and EXOPP vs. HF and PP) and polyphenols supplementation versus placebo (PP and EXOPP vs. HF and EXO) were performed using two-way analysis of variance with a Bonferroni *post hoc* test of pairwise comparisons between groups, or the Student's unpaired, two-tailed *t* test, where appropriate. A probability value of $<.$ 05 was considered significant.

2.10. Data availability

All data generated or analyzed during this study are included in this published article.

3. Results

3.1. Grape polyphenols and exercise improve metabolic and cardiovascular parameters in obese IR rats

After 1 month of high-fat diet, rats were obese and IR [40,41]. Consecutively, rats were supplemented with grape polyphenols in nutritional dose for 8 weeks with and without exercise while continuing the high-fat diet. At the end of the intervention, EXO and EXOPP rats were thinner than HF and PP rats (Table 1). As previously shown [40,41], an improvement of IR with exercise training was observed as HOMA-IR decreased (Table 1). Accordingly, EXOPP rats presented lower values of HOMA-IR indicating greater insulin

sensitivity compared with HF. However, only polyphenols supplementation decreased systolic blood pressure ($P=.$ 015). We found an interaction between polyphenols supplementation and exercise training on heart rate (Table 1). EXO rats presented a significant decrease in heart rate ($P=.$ 034) compared to HF but EXOPP rats had a significantly higher heart rate ($P=.$ 017) than EXO rats.

3.2. Grape polyphenols supplementation and exercise induce cardiac hypertrophy and reduce fibrosis

After 8 weeks of grape polyphenols supplementation at nutritional doses, we observed no differences between groups for heart and body weights (Table 1). We, however, observed an increase in the heart weight/body weight (HW/BW) ratio reflecting cardiac hypertrophy, in supplemented rats (PP and EXOPP) compared to nonsupplemented rats (HF and EXO; $P=.$ 004; Fig. 1A). There was an interaction between polyphenols supplementation and exercise ($P=.$ 041) in the HW/BW ratio and PP rats presented the highest HW/BW ratio with a large difference compared to HF rats ($P<.$ 001). Exercise tended to induce cardiac hypertrophy compared to the HF group ($P=.$ 056), while the EXOPP group was significantly different from HF ($P=.$ 002). Compared to HF rats, PP rats presented a 12% increase in the HW/BW ratio whereas EXO and EXOPP presented a 6% and 8% increase, respectively.

Since cardiomyocyte enlargement and fibrosis are hallmarks of cardiac hypertrophy remodeling, we then determined the cardiomyocytes size and collagen deposition. CSA (Fig. 1B and C) and Feret diameter (Fig. 1D) were increased after polyphenols supplementation ($P=.$ 022 and $P=.$ 027) and with exercise training ($P<.$ 001 and $P=.$ 008), without cumulative increase in EXOPP compared to the three other groups (Fig. 1C and D). Polyphenols supplementation decreased fibrosis ($P=.$ 014; Fig. 2A and B) and the effect of exercise training was close to significance ($P=.$ 091). There was an interaction between polyphenols supplementation and exercise training ($P<.$ 001). In fact, PP rats presented the lowest amount of fibrosis staining and tended to be lower than EXOPP ($P=.$ 065; Fig. 2B). To deepen our understanding of the intervention on the collagen structure, we determined unfolded/denatured collagen using a fluorescent peptide (R-CHP) able to specifically hybridize with denatured collagen (Fig. 2A and C). Polyphenols supplementation decreased R-CHP fluorescent intensity illustrating a smaller proportion of denatured collagen in PP and EXOPP compared to HF and EXO. The effect of exercise training was, as for fibrosis, close to significance ($P=.$ 086) with a decrease R-CHP intensity in exercising rats. Our R-CHP data demonstrate that the fibrosis observed was mainly the consequences of

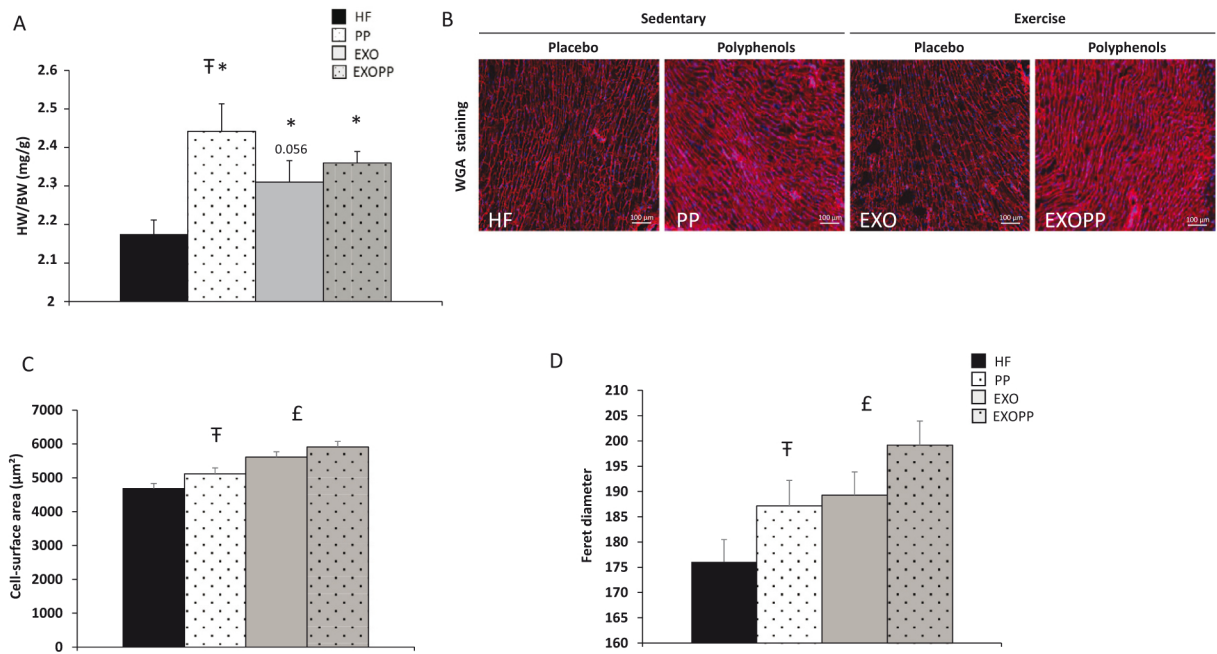


Fig. 1. Grape polyphenols supplementation and exercise induce cardiac hypertrophy. (A) Heart weight on body weight ratio (HW/BW). (B) Representative staining of cardiac sections performed with DAPI (blue) for nuclei and with WGA for the plasma membrane (red). (C) Cell-surface area of cardiomyocyte labeled with WGA as shown in B. (D) Feret diameter of cardiomyocytes labeled with WGA. Data are expressed as mean \pm SEM with $n=8-10$ rats per group. £ effect of exercise, ‡ effect of grape supplementation, * $P<.05$ versus HF.

unfolded/denaturated collagen with a beneficial effect of polyphenols supplementation without further improvement of adding exercise.

3.3. Grape polyphenols and exercise mediate cardiac hypertrophy via distinct pathways

We then investigated the physiological and pathologic pathways known to be involved in cardiac hypertrophy. P-Akt/akt ratio was increased with exercise training but was not modified by polyphenols supplementation (Fig. 3A). Calcineurin expression was decreased by both exercise and polyphenols supplementation with a significant interaction between the two conditions ($P=.008$, Fig. 3B). The effect of exercise and supplementation on calcineurin was not additive; hence, while both exercising groups were lower than PP, there was no significant difference between EXO and EXOPP.

3.4. Grape polyphenols supplementation and exercise alter cardiac metabolism distinctly

Since metabolism is essential for cardiac hypertrophy development, we sought to investigate AMPK activation as it is known to be involved in the activation of energy-generating pathways and inhibition of energy-consuming pathways. We found higher P-AMPK/AMPK ratio in exercise training rats compared to sedentary rats (EXO, EXOPP vs. HF, PP; Fig. 4A and B). PP rats also presented a tendency for increased P-AMPK/AMPK ratio compared to HF ($P=.077$). We then determined heart oxidative capacity by measuring citrate synthase activity. Citrate synthase activity was increased ($P=.049$, Fig. 4C) in exercising rats compared to sedentary rats, with no effect of supplementation. In contrast, lipid oxidation, assessed with hydroxyacylCoA dehydrogenase activity (HADH), was not modified by the experimental conditions (Fig. 4D).

3.5. Grape polyphenols supplementation and exercise alter Ca²⁺ handling-related proteins distinctly

Since Ca²⁺ handling-related proteins contribute to cardiac function, we investigated whether the expression of these proteins was altered in exercise and polyphenols supplementation. PP and EXO rats increased SERCA protein expression ($P=.048$ and $P=.015$, in PP and EXO compared to HF respectively; Fig. 5A) but not when combined, as EXOPP rats were not significantly different from HF rats ($P=.095$). There was an interaction ($P=.050$) between exercise and polyphenols supplementation. The SERCA activity depends on the phosphorylation level of PLN. We further investigated the phosphorylation of PLN at Thr₁₇ by -calmodulin and Ca²⁺ dependent kinase (Fig. 5B). The P-PLN_{Thr17} /PLN ratio was increased by polyphenols supplementation (PP, EXOPP vs. HF, EXO; $P=.04$) due to a large increase in P-PLN_{Thr17} /PLN ratio in PP rats, with no significant effect of exercise. Finally, the SERCA/PLN ratio was increased in all group compared to HF (Fig. 5C). Only EXO rats presented a significant difference ($P=.012$ vs. HF) although PP rats approached significance ($P=.062$). Again, there was no benefit of combining exercise and polyphenols supplementation on this parameter.

4. Discussion

In this study, we demonstrated that a grape polyphenols supplementation at nutritional dose or exercise training for 8 weeks distinctly altered molecular signaling in the heart of obese insulin-resistant rats. The combination of both interventions did not, however, further improve the benefits of individual intervention.

As previously reported [40,41], 8 weeks of exercise led to significant improvements in body composition. Exercise limited weight gain, increased HDL-cholesterol and reduced IR, thereby lowering CVDs risk compared to sedentary rats. Moreover, the combination of exercise and nutritional grape polyphenols supplementation had a synergistic effect on endurance exercise capacity by increasing muscle lipid oxidation and sparing glycogen utilization

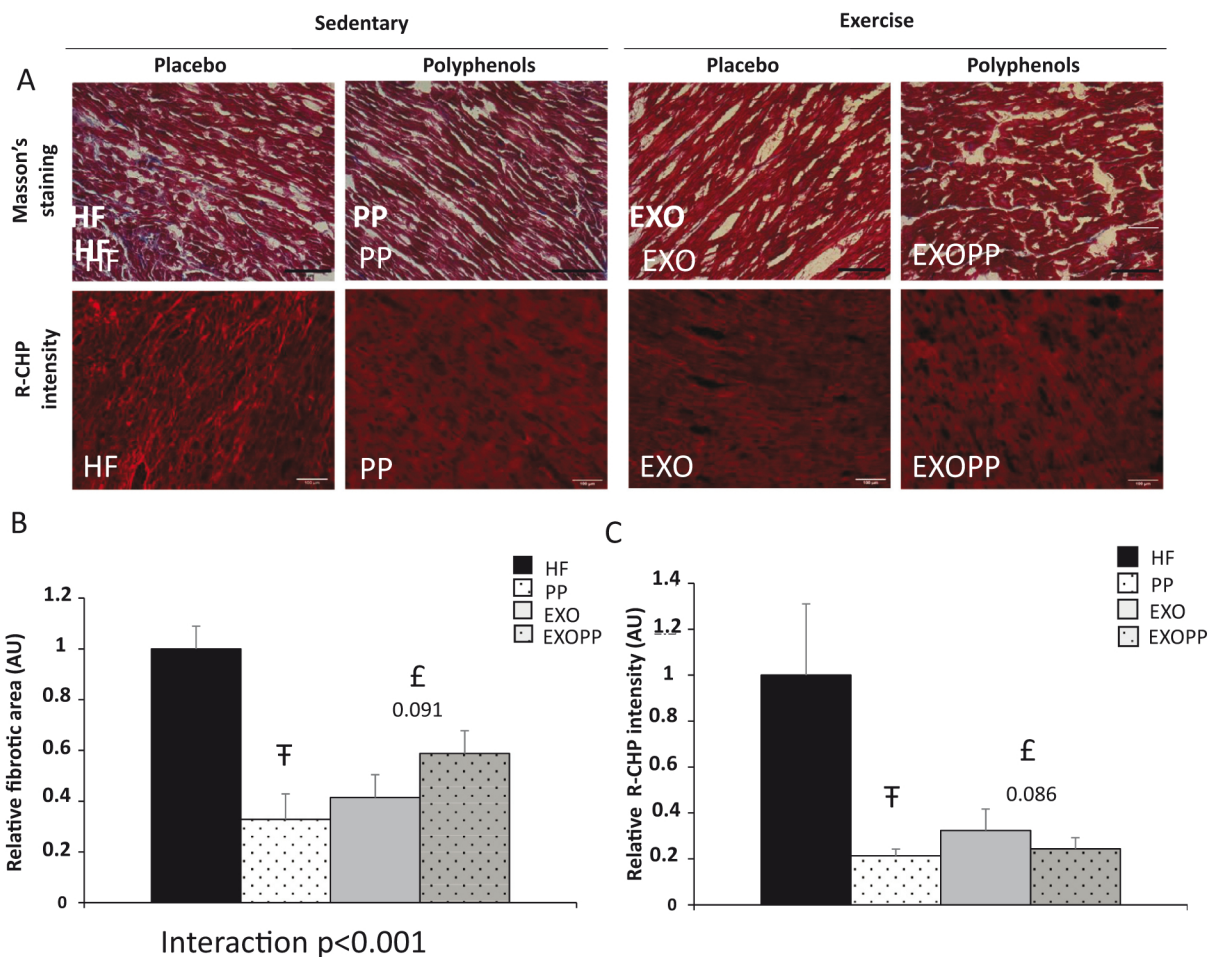


Fig. 2. Grape polyphenols supplementation and exercise reduce fibrosis. **(A)** Representative staining of cardiac sections performed with Masson's trichrome (upper part) illustrating fibrosis (blue), cytosol (red), and Collagen Hybridizing Peptide, Cy3 Conjugate (R-CHP) (lower part) illustrating denaturated collagen (red fluorescence). **(B)** Relative fibrosis and **(C)** relative R-CHP intensity quantifications are expressed relative to HF value, which was set at 1. Data are expressed as mean \pm SEM with at least three fields per rat and three rats per group. £ effect of exercise, F effect of grape supplementation.

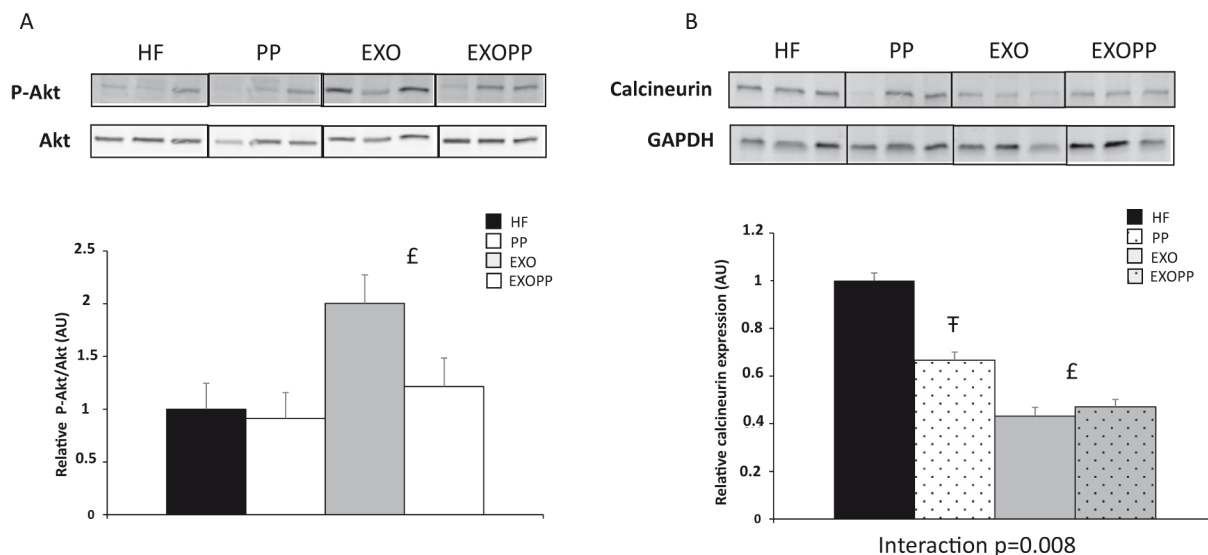


Fig. 3. Grape polyphenols and exercise mediate cardiac hypertrophy via distinct pathways. **(A)** P-Akt, Akt expression levels measured by immunoblotting. A representative blot of three rats by group is shown. Quantification values are represented as P-Akt/Akt ratio and are expressed relative to HF value, which was set at 1. **(B)** Calcineurin expression levels measured by immunoblotting. A representative blot of three rats by group is shown. Quantification values are expressed relative to HF value, which was set at 1. Data are expressed as mean \pm SEM with $n=5-8$ rats per group. £ effect of exercise, F effect of grape supplementation.

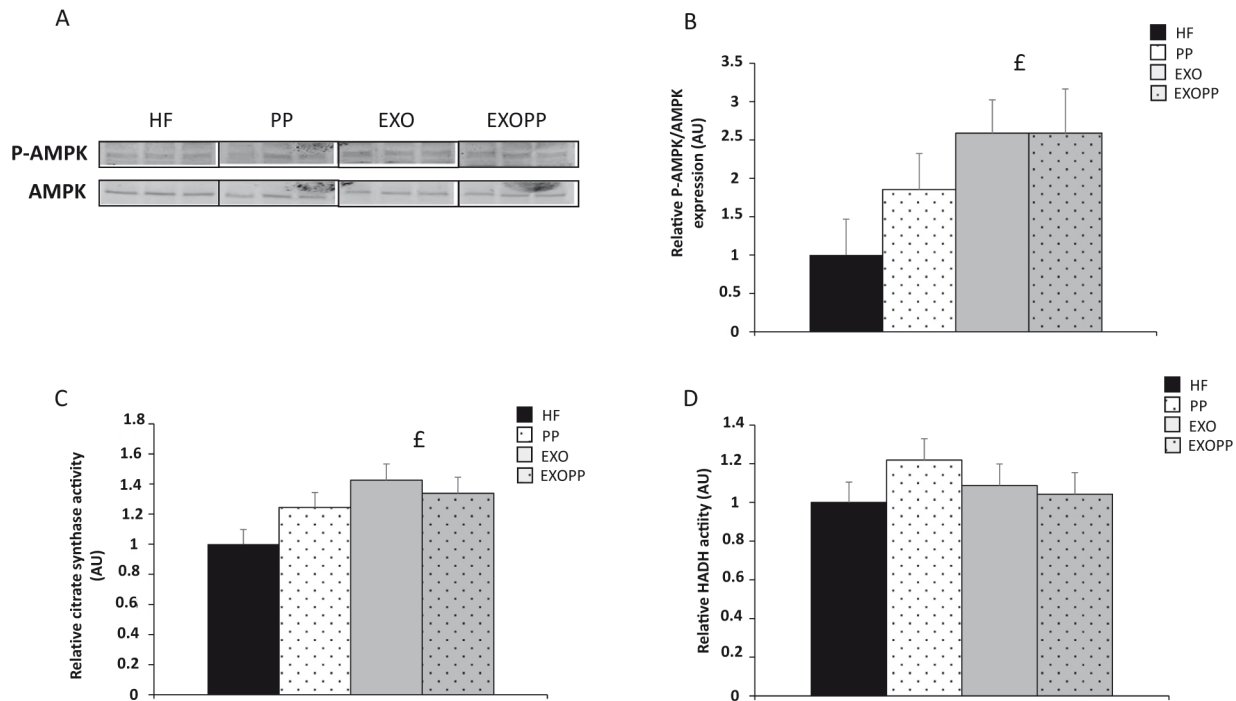


Fig. 4. Grape polyphenols supplementation and exercise alter cardiac metabolism distinctly. **(A)** P-AMPK, AMPK expression levels measured by immunoblotting. A representative blot of three rats by group is shown. **(B)** P-AMPK, AMPK quantification values are represented as P-AMPK/AMPK ratio and are expressed relative to HF value, which was set at 1. **(C)** Mitochondrial citrate synthase and **(D)** hydroxyacylCoA dehydrogenase (HADH) activities are expressed relative to HF value, which was set at 1. Data are expressed as mean \pm SEM with $n=5-8$ rats per group. £ effect of exercise.

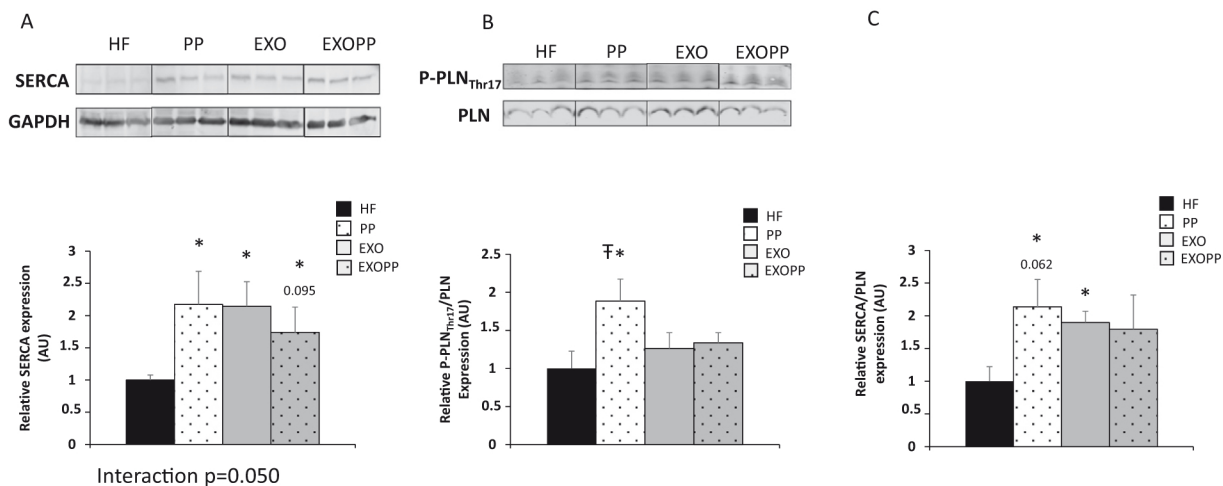


Fig. 5. Grape polyphenols supplementation and exercise alter calcium handling protein distinctly. **(A)** SERCA expression levels measured by immunoblotting. A representative blot of three rats by group is shown. Quantification values are expressed relative to HF value, which was set at 1. **(B)** P-PLN_{Thr17}, PLN expression levels measured by immunoblotting. A representative blot of three rats by group is shown. Quantification values are represented as P-PLN_{Thr17}/PLN ratio and are expressed relative to HF value, which was set at 1. **(C)** Relative SERCA/PLN expression are expressed relative to HF value, which was set at 1. Data are expressed as mean \pm SEM with $n=5-8$ rats per group. £ effect of exercise, ¶ effect of grape supplementation, * $P<.05$ versus HF.

in skeletal muscle [40]. In this present work, we went further in the characterization of the beneficial effects of exercise and grape polyphenols supplementation by analyzing heart metabolism.

Exercise training induced a modest (6 and 8%) but significant increase in HW/BW ratio in EXO and EXOPP compared to HF. These results are consistent with the mild (10%–20%) exercise-induced increase in cardiac size of highly trained athletes [16]. Exercised rats favored a physiological cardiac hypertrophy as attested by the increased P-Akt/Akt ratio. This ratio, ranging from 1.21 to 2, is in agreement with the range of the physiological activation previously described after exercise training [14]. Activation of the

Akt signaling pathway has been shown to be protective in this range whereas higher expression (>15 fold) promoted pathologic hypertrophy [46]. Moreover, we also found a significant increase in P-AMPK/AMPK ratio only in exercised-rats (EXO and EXOPP rats). In lean endurance-trained rats, Dolinsky et al. also found an increased P-AMPK/AMPK ratio, which was positively associated with citrate synthase activity [39]. In our study, citrate synthase activity was similarly increased only in exercised rats without alteration in lipid oxidation. The heart metabolism is characterized by a high oxidative capacity as most of its ATP requirement ($\approx 95\%$) is provided by oxidative phosphorylation, while the remaining 5% is de-

rived from glycolysis [47]. To support its important energy needs, the heart is able to use both fatty acids and glucose which compete for catabolism [47]. Thus, metabolism alteration is considered as an essential trigger for cardiac hypertrophy [16,48]. Since exercise has led to cardiac hypertrophy, there was an increase in ATP requirement provided by mitochondrial activity and AMPK activation. Importantly, calcineurin expression, reflecting the pathologic signaling pathway, is decreased by 60% in exercised rats. This signaling pathway is not associated with physiological cardiac hypertrophy [49]. Cardiac fibrosis is often observed in obesity, especially with abdominal adiposity [9]. Exercise induced a large reduction in fibrosis (~50%) associated with lower adiposity [41]. Thus, exercise in obese IR rat favors the physiological cardiac hypertrophy signaling while decreasing the pathologic one. As opposed to a previous study on resveratrol in lean rats [39], our grape polyphenols mixture did not have a synergistic effect on the parameters tested in this study. Moreover, on some parameters such as heart rate or P-Akt/Akt ratio, polyphenols supplementation associated with exercise training impeded the beneficial effect of exercise. However, polyphenols supplementation was able to significantly decrease systolic blood pressure, which could explain the beneficial effect of polyphenols observed in some studies on CVDs [36].

In the sedentary condition, polyphenols supplementation induced cardiac hypertrophy—a higher HW/BW ratio—through a modest increase in CSA. Polyphenols supplementation did not, however, increase the canonical physiological signaling pathway P-Akt/Akt or P-AMPK/AMPK ratios. Possible explanations for the increased HW/BW ratio without a concomitant elevation in P-Akt/Akt ratio could reside in a reduced apoptosis, as previously found in heart of exercising obese rats [50]. Nevertheless, PP rats presented a 40% decrease in calcineurin expression compared to HF rats. Since the activation of calcineurin/NFAT signaling alone is sufficient to induce pathologic cardiac hypertrophy [49,51] a large reduction in its expression could result in a shift toward physiological hypertrophy. In accordance, PP rats presented highest reduction in fibrosis and denatured collagen. The reduction of calcineurin expression, fibrosis, and denatured collagen highlights an improvement of cardiac physiology and possibly contractile function. In this regard, the increases in SERCA protein expression and P-PLN_{Thr17}/PLN ratio in PP would increase SERCA activity and Ca²⁺ reuptake by the sarcoplasmic reticulum (SR) [7]. An enhanced SR Ca²⁺ transport increases the rate of decay of the Ca²⁺ transient and the Ca²⁺ load on the SR. This improves relaxation and contraction functions particularly during aerobic exercise. Accordingly, EXO rats exhibited an increase only for SERCA protein expression and SERCA/PLN ratio [52].

In conclusion, we herein provide new data regarding (1) the cardiac metabolic changes that occur following exercise training in obese IR rats, (2) the effect of a nutritional dose of a grape polyphenols mixture as a treatment for cardiac alteration since supplementation began after obesity and IR, (3) the individual and combined effect of exercise and polyphenols supplementation on cardiac physiological hypertrophy.

Our study demonstrates that polyphenols supplementation has physical-activity-status-specific effects and appears to be more potent in sedentary obese IR rats than in the exercised ones. Exercise training improved metabolic and cardiac alterations without a synergistic effect of polyphenols supplementation. These data highlight a greater effect of exercise than polyphenols supplementation for the treatment of cardiac alterations in obese IR rats.

Conflict of interest

The authors declare that they have no conflicts of interest.

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