Positive effect of combined exercise training in a model of metabolic syndrome and menopause: autonomic, inflammatory, and oxidative stress evaluations

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[1]*Laboratory of Translational Physiology, Universidade Nove de Julho (UNINOVE), São Paulo, Brazil;* [2]*Hypertension Unit, Heart Institute (InCor), School of Medicine, University of Sao Paulo, São Paulo, Brazil; and* [3]*Institute of Neuro-Immune Medicine, Nova Southeastern University, Fort Lauderdale, Florida; and* [4]*Universidad de Buenos Aires, Buenos Aires, Argentina*

Conti FF, Brito JO, Bernardes N, Dias DS, Malfitano C, Morris M, Llesuy SF, Irigoyen MC, De Angelis K. Positive effect of combined exercise training in a model of metabolic syndrome and menopause: autonomic, inflammatory, and oxidative stress evaluations. *Am J Physiol Regul Integr Comp Physiol* 309: R1532–R1539, 2015. First published September 22, 2015; doi:10.1152/ajpregu.00076.2015.—It is now well established that after menopause cardiometabolic disorders become more common. Recently, resistance exercise has been recommended as a complement to aerobic (combined training, CT) for the treatment of cardiometabolic diseases. The aim of this study was to evaluate the effects of CT in hypertensive ovariectomized rats undergoing fructose overload in blood pressure variability (BPV), inflammation, and oxidative stress parameters. Female rats were divided into the following groups ($n = 8$/group): sedentary normotensive Wistar rats (C), and sedentary (FHO) or trained (FHOT) ovariectomized spontaneously hypertensive rats undergoing and fructose overload. CT was performed on a treadmill and ladder adapted to rats in alternate days (8 wk; 40–60% maximal capacity). Arterial pressure (AP) was directly measured. Oxidative stress and inflammation were measured on cardiac and renal tissues. The association of risk factors (hypertension + ovariectomy + fructose) promoted increase in insulin resistance, mean AP (FHO: 174 ± 4 vs. C: 108 ± 1 mmHg), heart rate (FHO: 403 ± 12 vs. C: 352 ± 11 beats/min), BPV, cardiac inflammation (tumor necrosis factor-α-FHO: 65.8 ± 9.9 vs. C: 23.3 ± 4.3 pg/mg protein), and oxidative stress cardiac and renal tissues. However, CT was able to reduce mean AP (FHOT: 158 ± 4 mmHg), heart rate (FHOT: 303 ± 5 beats/min), insulin resistance, and sympathetic modulation. Moreover, the trained rats presented increased nitric oxide bioavailability, reduced tumor necrosis factor-α (FHOT: 33.1 ± 4.9 pg/mg protein), increased IL-10 in cardiac tissue and reduced lipoperoxidation, and increased antioxidant defenses in cardiac and renal tissues. In conclusion, the association of risk factors promoted an additional impairment in metabolic, cardiovascular, autonomic, inflammatory, and oxidative stress parameters and combined exercise training was able to attenuate these dysfunctions.

exercise training; menopause; metabolic syndrome; blood pressure variability; inflammation; oxidative stress

AFTER MENOPAUSE, hypertension, insulin resistance, Type 2 diabetes, and morbidity are all increased. These changes are impaired with increased intake of simple sugars, particularly fructose, commonly used in the food industry and sugar-sweetened drinks (33). Moreover, the association between autonomic dysfunction and cardiovascular diseases strengthens evidence that alterations in the sympathovagal control increase cardiovascular risk after menopause (32). Furthermore, there seems to be an association between oxidative stress and inflammation, and both would play a role in the development of several cardiovascular diseases (26).

Indeed, several studies have pointed to the adverse cardiac and metabolic effects of a high-fructose diet in animal models (15, 17, 20, 29, 44). Our group has demonstrated that only 8 wk consumption of fructose was sufficient to induce prominent metabolic, cardiovascular, and renal changes associated with autonomic dysfunctions in mice and rats (8, 20, 38, 44). Recently, we (17) demonstrated that increased sympathetic modulation for vessels and heart preceded the development of metabolic dysfunction in fructose fed mice.

On the other hand, aerobic exercise training has been recommended as an important adjuvant therapy for the prevention and treatment of cardiometabolic disorders. Our group has demonstrated that aerobic exercise training in spontaneously hypertensive ovariectomized rats attenuated and/or prevented increase in blood glucose and triglyceride concentrations, reduced insulin resistance induced by fructose overload, and also improved cardiovascular autonomic modulation in this condition (44).

Recently, resistance exercise training has been recommended by several clinical guidelines as a complement to aerobic exercise (combined training) as an effective nonpharmacological treatment tool in cardiovascular and metabolic diseases (40, 49, 50). However, the role of combined exercise training on cardiovascular control, inflammation, and oxidative stress has yet to be fully understood. Thus our study was designed to evaluate the hypothesis that combined exercise training may reduce and/or revert injuries promoted by fructose overload on cardiovascular and renal system in hypertensive rats undergoing ovarian hormone deprivation. Therefore, the aim of this study was to evaluate the effects of combined exercise training in hypertensive ovariectomized rats undergoing fructose overload on cardiovascular autonomic modulation, inflammation, and oxidative parameters.

METHODS

Females spontaneously hypertensive rats (SHR) and Wistar rats, 21 days old, were obtained from the Animal Facility of the Institute of Cardiology of Rio Grande do Sul. The rats were randomly assigned as

follows: sedentary normotensive Wistar rats (C; $n = 8$), sedentary ovariectomized SHR undergoing fructose overload (FHO; $n = 8$), and trained ovariectomized SHR undergoing fructose overload (FHOT; $n = 8$). All surgical procedures and protocols were approved by the ethics committee of Universidade Nove de Julho (Protocol AN 002-12) and were conducted in accordance with National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

Fructose Overload

FHO and FOHT rats received D-fructose (100 g/l) in the drinking water for 19 wk. C animals received standard laboratory chow and water ad libitum.

Ovariectomy

After 10 wk of fructose overload, the SHR were anesthetized (80 mg/kg ketamine and 12 mg/kg xylazine), the oviduct was sectioned, and the ovary was removed as described in detail elsewhere (21, 28). Data from our laboratory have previously demonstrated that estrogen concentration, measured through immunoassay, was 39 ± 7 pg/ml in intact female rats. However, in the present study, estrogen concentrations on the plasma were not detected in ovariectomized studied groups, thus confirming ovarian hormone deprivation (21).

Combined Exercise Training

Combined exercise training was performed on a motor treadmill (aerobic training) and in a ladder adapted to rats (resistance training), in alternate days, 5 days/wk, for 8 wk. For accuracy of prescription, maximal (running or load) tests were performed at the beginning of the experiment and in the 4th and 8th wk of the training protocol.

Aerobic exercise training. All animals were adapted to walk and run on a motorized treadmill (10 min/day; 0.3 km/h) for 5 consecutive days, before the maximal running test. The aerobic exercise test was performed in sedentary and trained rats as described in detail in a previous study (43). Aerobic exercise training was performed on a treadmill (Imbramed TK-01, Brazil) at low-to-moderate intensity ($\sim 40-60\%$ maximal running speed) for 1 h a day, in alternate days with resistance exercise training, for 8 wk. To provide a similar environment and manipulation, sedentary animals were placed on the stationary treadmill three times a week.

Resistance exercise training. The animals were gradually adapted to the act of climbing for 5 consecutive days before the maximal load test. This is a voluntary exercise protocol, with no aversive (electrical) stimuli to maintain performance, no restraint, and no use of food or water as motivators. The dynamic resistance exercise test was composed of an initial load of 75% of the body weight. After a 2-min resting period, a gradual increase of 15% of body weight was used in the subsequent climbs, as previously described in detail elsewhere (45). The prescription of resistance exercise training was performed using the normalized value of maximal load for each rat and was adjusted weekly, according to the body weight of the animal. The resistance exercise training protocol was performed during 8 wk, alternating days with aerobic exercise training at low-moderate intensity (1st-2nd wk: 30–40%; 3th-5th wk: 40–50%; 6th-8th wk: 40–60% of the maximal load) with 15 climbs per session and a 1-min time interval between climbs as previously described in details elsewhere (45). Importantly, to maintain the standard of 6 climbs for maximal load, load increment was adjusted in the maximal load test performed on the 4th and 8th wk of the protocol, with +25% and +40% of the body weight increments between climbs, respectively. The purpose was to determine physical capacity and exercise training intensity (14).

Metabolic Evaluations

The insulin tolerance test was performed at the end of exercise protocol (19 wk of fructose overload). The animals fasted for 2 h and were then anesthetized with thiopental sodium (40 mg/kg body wt ip). A drop of blood was collected from the tail for blood glucose level measurement at baseline and 4, 8, 12, and 16 min after insulin injection (0.75 U/kg). The constant rate for blood glucose disappearance (K_{itt}) was calculated using the $0.693/t_{1/2}$ formula. Blood glucose $t_{1/2}$ was calculated from the slope of the least squares analysis of blood glucose concentrations during the linear phase of decline (6, 8).

Cardiovascular Measurements

After the last training session, two catheters filled with 0.06 ml saline were implanted in anesthetized rats (80 mg/kg ketamine and 1 mg/kg xylazine) into the carotid artery and jugular vein (PE-10) for direct measurements of AP and drug administration, respectively. Rats receiving food and water ad libitum were studied 1 day after catheter placement; they remained conscious and were allowed to move freely during the experiments. An arterial cannula was connected to a transducer (Blood Pressure XDCR, Kent Scientific), and blood pressure signals were recorded over a 20-min period by a microcomputer equipped with an analog-to-digital converter board (Windaq, 2-kHz sampling frequency; Dataq Instruments). The recorded data were analyzed on a beat-to-beat basis to quantify changes in mean arterial pressure (MAP) and heart rate (HR). Increasing doses of phenylephrine (0.25 to 32. μg/kg)and sodium nitroprusside (0.05 to 1.6 μg/kg) were given as sequential bolus injections (0.1 ml/dose) to produce pressure responses ranging from 5 to 40 mmHg. A 3- to 5-min interval between doses was necessary for blood pressure to return to baseline. Peak increases or decreases in MAP after phenylephrine or sodium nitroprusside injection and the corresponding peak reflex changes in HR were recorded for each dose of the drug. Baroreflex sensitivity (BRS) was evaluated by a mean index relating changes in HR to changes in MAP, allowing a separate analysis of gain for reflex bradycardia and reflex tachycardia. The mean index was expressed as beats per minute per millimeter of mercury, as described elsewhere (21, 28, 44).

Autonomic Measurements

Time-domain analysis consisted of calculating systolic arterial pressure (SAP) variability measured from its respective time series (three time series of 5 min for each animal). For frequency domain analysis, the same time series of SAP was cubic spline Y interpolated (250 Hz) and cubic spline Y decimated to be equally spaced in time after linear trend removal; power spectral density was obtained through the fast-Fourier transformation. Spectral power for low-frequency (LF; 0.20–0.75 Hz) and high-frequency (HF; 0.75–4.0 Hz) bands were calculated by power spectrum density integration within each frequency bandwidth, using a customized routine (MATLAB 6.0, Math works) (46).

Determination of NO Metabolites

Nitrites (NO_2) were determined in plasma using the Griess reagent in which a chromophore with a strong absorbance at 540 nm is formed by the reaction of nitrite with a mixture of naphthylethylenediamine (0.1%) and sulfanilamide (1%). Nitrates (NO_3) were determined as the total of nitrites (initial nitrite plus nitrite reduced from nitrate) after (its) reduction using nitrate reductase from *Aspergillus* species in the presence of DUOX1. A standard curve was established with a set of serial dilutions (10^{-18} to 10^{-13} mol/l) of sodium nitrite. Results were expressed as millimole per milligram protein of nitrates plus nitrites (23). Nitric oxide (NO) metabolites (NOx) were calculated by the sum of plasma nitrate and nitrite.

Inflammatory Markers on Cardiac Tissue

One day after hemodynamic evaluations, the animals were killed and the heart (ventricles) was immediately removed, rinsed in saline, and trimmed to remove fat tissue and visible connective tissue. IL-6,

IL-10, and tumor necrosis factor-α (TNF-α) levels were determined using a commercially available ELISA kit (R&D Systems), in accordance with the manufacturer's instructions. ELISA was performed in 96-well polystyrene microplates with a specific monoclonal antibody coating. The threshold of sensitivity for the TNF-α, IL-10, and IL-6 assays was 15.0 pg/ml. Absorbance was measured at 540 nm in a microplate reader.

Oxidative Stress Profile on Cardiac and Renal Tissues

The cardiac tissue (left ventricle) ($\sim$0.5 g) and whole kidney were cut into small pieces, placed in an ice-cold buffer, and homogenized in an ultra-Turrax blender with 1 gram tissue per 5 ml, 150 mM KCl, and 20 nM sodium phosphate buffer, pH 7.4. The homogenate was centrifuged at 600 g for 10 min at $-26°$C.

Hydrogen peroxide concentration. The assay was based on the horseradish peroxidase (HRPO)-mediated oxidation of phenol red by H_2O_2, leading to the formation of a compound measurable at 610 nm. Heart homogenates were incubated for 30 min at 37°C in 10 mmol/l phosphate buffer consisting of 140 mmol/l NaCl and 5 mmol/l dextrose. The supernatants were transferred to tubes with 0.28 mmol/l phenol red and 8.5 U/ml HRPO. After 5 min incubation, 1 mol/l NaOH was added and it was read at 610 nm. The results were expressed in nanomol H_2O_2 per gram tissue (39).

Membrane lipoperoxidation by chemiluminescence and thiobarbituric acid reactive substances. The chemiluminescence (CL) assay was carried out with an LKB Rack Beta liquid scintillation spectrometer 1215 (LKB Producer) in the out-of-coincidence mode at room temperature. Supernatants were diluted in 140 mM KCl and 20 mM sodium phosphate buffer, pH 7.4, and added to glass tubes, which were placed in scintillation vials; 3 mM tert-butyl hydroperoxide was added, and CL was determined up to the maximal level of emission (22, 28). For thiobarbituric acid reactive substances (TBARS) assay, trichloroacetic acid (10%, wt/vol) was added to the homogenate to precipitate proteins and to acidify the samples (9). This mixture was then centrifuged (3,000 g, 3 min), the protein-free sample was extracted, and thiobarbituric acid (0.67%, wt/vol) was added to the reaction medium. The tubes were placed in a water bath (100°C) for 15 min. The absorbances were measured at 535 nm using a spectrophotometer. Commercially available malondialdehyde (MDA) was used as a standard, and the results are expressed as micromole per milligram of protein.

Determination of protein oxidation using the carbonyls assay. Tissue samples were incubated with 2,4-dinitrophenylhydrazine (DNPH 10 mM) in a 2.5 M HCl solution for 1 h at room temperature in the dark. Samples were vortexed every 15 min. Subsequently, a 20% trichloroacetic acid (wt/vol) solution was added and the solution was incubated on ice for 10 min and centrifuged for 5 min at 1,000 g to collect protein precipitates. An additional wash was performed with 10% trichloroacetic acid (wt/vol). The pellet was washed three times with ethanolethyl acetate (1:1) (vol/vol). The final precipitates were dissolved in 6 M guanidine hydrochloride solution and incubated for 10 min at 37°C, and the absorbance was measured at 360 nm (42).

Antioxidant enzyme activities. The quantification of superoxide dismutase (SOD) activity, expressed as units SOD per milligram protein, was based on the inhibition of the reaction between $O_2^{\cdot-}$ and pyrogallol (37). Catalase (CAT) activity was determined by measuring the decrease in H_2O_2 absorbance at 240 nm. [CAT activity was expressed as μmol H_2O_2 reduced·min^{-1}·mg protein^{-1} (1)].

Glutathione and GSSG concentration. To determine GSSG and total glutathione concentration, tissue was homogenized in 2 M perchloric acid and centrifuged at 1,000 g for 10 min, and 2 M KOH was added to the supernatant. The reaction medium contained 100 mM phosphate buffer (pH 7.2), 2 mM NADPH, 0.2 U/ml glutathione reductase, and 70 μM 5,5′-dithiobis(2-nitrobenzoic acid). To determine GSSG concentration, the supernatant was neutralized with 2 M KOH and inhibited by the addition of 5 μM N-ethylmaleimide.

Absorbance was read at 420 nm (3). GSH values were determined from the total glutathione and GSSG concentrations.

TRAP. Total antioxidant capacity (TRAP) was measured using 2,2-azo-bis(2-amidinopropane) (ABAP, a source of alkyl peroxyl free radicals) and luminol. A mixture consisting of 20 mmol/l ABAP, 40 μmol/l luminol, and 50 mmol/l phosphate buffer (pH 7.4) was incubated to achieve a steady-state luminescence from the free radical-mediated luminol oxidation. A calibration curve was obtained by using different concentrations (between 0.2 and 1 μmol/l) of Trolox (hydrosoluble form of vitamin E). Luminescence was measured in a liquid scintillation counter using the out-of-coincidence mode, and the results were expressed in units of Trolox per milligram protein (18).

Statistical analysis. Data are expressed as means $\pm$ SE. The Levene test was used to evaluate data homogeneity. A one-way analysis of variance followed by the Student-Newman-Keuls test was used to compare groups. Significance level was established at $P \le 0.05$.

RESULTS

The FHOT group showed a higher maximal aerobic and load capacity compared with sedentary groups at the end of the protocol (Table 1).

The body weight was similar between C and FHO groups. The FHOT group presented reduced body weight compared with C and FHO groups. Triglyceride levels were increased and insulin sensitivity (K_{itt}) was reduced in FHO group compared with C group. The combined exercise training was able to normalize these dysfunctions (FHOT vs. FHO) (Table 1).

Heart rate was higher in the FHO group compared with C group (FHO: 403 $\pm$ 12 vs. C: 352 $\pm$ 11 beats/min). The FHOT group presented a resting bradycardia (FHOT: 303 $\pm$ 5 beats/min) (Fig. 1*B*). MAP was higher in the FHO group compared with C group (FHO: 174 $\pm$ 4 vs. C: 108 $\pm$ 1 mmHg) (Fig. 1*A*). The FHOT group (158 $\pm$ 4 mmHg) showed decreased MAP compared with the FHO group (Fig. 1*A*). SAP variance (VAR-SAP) and LF-SAP were higher in FHO group compared with all other groups; however, combined exercise training promoted a normalization of these parameters (FHOT vs. C) (Fig. 1, *C* and *D*).

Baroreflex sensitivity was reduced in the fructose groups (FHO and FHOT) compared with the C group, for both bradycardic responses (FHO: 1.01 $\pm$ 0.15; FHOT: 1.11 $\pm$ 0.08 vs. C: -1.52 ± 0.08 bpm/mmHg) and tachycardic responses (FHO: 1.17 $\pm$ 0.12; FHOT: 1.59 $\pm$ 0.10 vs. C: 4.37 $\pm$ 0.34 bpm/mmHg).

There was no difference in plasmatic NOx between FHO and C rats. However, NOx was increased in the FHOT group

Table 1. *Physical capacity and metabolic evaluations in studied groups*

	C	FHO	FHOT
Maximal aerobic test, min	18 $\pm$ 1.5	19 $\pm$ 1.0	22 $\pm$ 0.3*
Maximal resistance test, g	204 $\pm$ 11	467 $\pm$ 19*	523 $\pm$ 11*†
Body weight, g	266 $\pm$ 4	261 $\pm$ 2	253 $\pm$ 2*†
Triglycerides, mg/dl	125 $\pm$ 7	160 $\pm$ 8*	137 $\pm$ 4†
K_{itt}, %/min	4.42 $\pm$ 0.29	3.40 $\pm$ 0.28*	4.69 $\pm$ 0.34†

Data are expressed as means $\pm$ SE. C, sedentary normotensive Wistar rats; FHO, sedentary ovariectomized SHR undergoing fructose overload; FHOT, trained ovariectomized SHR undergoing fructose overload; K_{itt}, constant rate for blood glucose disappearance. *$P < 0.05$ vs. C; †$P < 0.05$ vs. FHO.
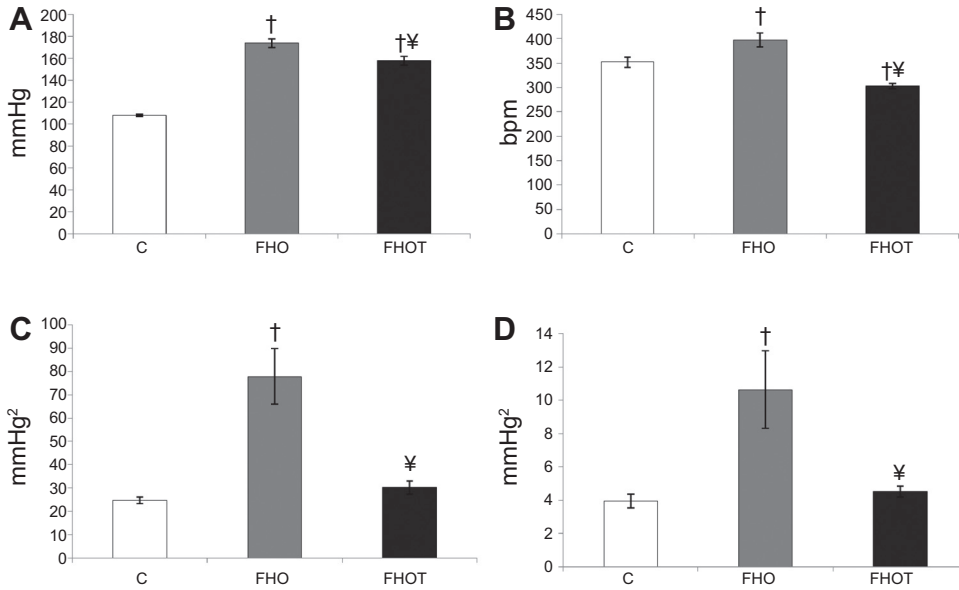
Fig. 1. *A*: mean arterial pressure; *B*: heart rate; *C*: systolic arterial pressure variance (SAP)-VAR; *D*: SAP-LF in all studied groups. C, sedentary normotensive Wistar rats; FHO, sedentary ovariectomized spontaneously hypertensive rats (SHR) undergoing fructose overload; FHOT, trained ovariectomized SHR undergoing fructose overload; †$P < 0.05$ vs. C; ¥$P < 0.05$ vs. FHO.

(3.88 ± 0.49 nmol/mg protein) compared with C group (1.22 ± 0.12 nmol/mg protein) and FHO group (1.82 ± 0.65 nmol/mg protein) (Fig. 2*A*).

TNF-α was higher in the FHO compared with C group (FHO: 65.8 ± 9.9 vs. C: 23.3 ± 4.3 pg/mg protein), but the combined exercise training normalized this parameter (FHOT: 33.1 ± 4.9 pg/mg protein) (Fig. 2*B*). IL-10 was lower in the FHO group (16.2 ± 2.5 pg/mg protein) compared with the C group (32.5 ± 4.0 pg/mg protein). No differences were observed in IL-10 between the C group and FHOT group (26.7 ± 5.7 pg/mg protein) (Fig. 2*C*).

Regarding oxidative stress in cardiac tissue, hydrogen peroxide was higher in FHO group compared with C group. No differences in cardiac hydrogen peroxide were observed between C and FHOT groups. Protein oxidation (carbonyls) was higher in both groups undergoing fructose overload (FHO and FHOT vs. C). Lipoperoxidation (CL) was higher in FHO

compared with C group. The FHOT group showed a reduction of lipoperoxidation compared with the FHO group. Chronic consumption of fructose promoted a reduction of cardiac CAT and did not change SOD activity or TRAP. Combined exercise training was able in to increase cardiac CAT activity compared with FHO group. SOD activity and TRAP were higher in the FHOT group compared with both sedentary groups (Table 2). Importantly, despite we did not observe significant changes in cardiac GSH/GSSG between FHO and C groups, the FHOT rats showed an increased GSH/GSSG compared with FHO rats, thus indicating improvement in redox balance (Fig. 2*D*).

Concerning oxidative stress in renal tissue, lipoperoxidation (TBARS) was higher in both FHO and FHOT groups compared with C group. Protein oxidation (carbonyls) was higher in the FHO group than in the C group, but combined exercise training was able to decrease carbonyls (FHOT vs. FHO). There was no difference in renal CAT activity among the
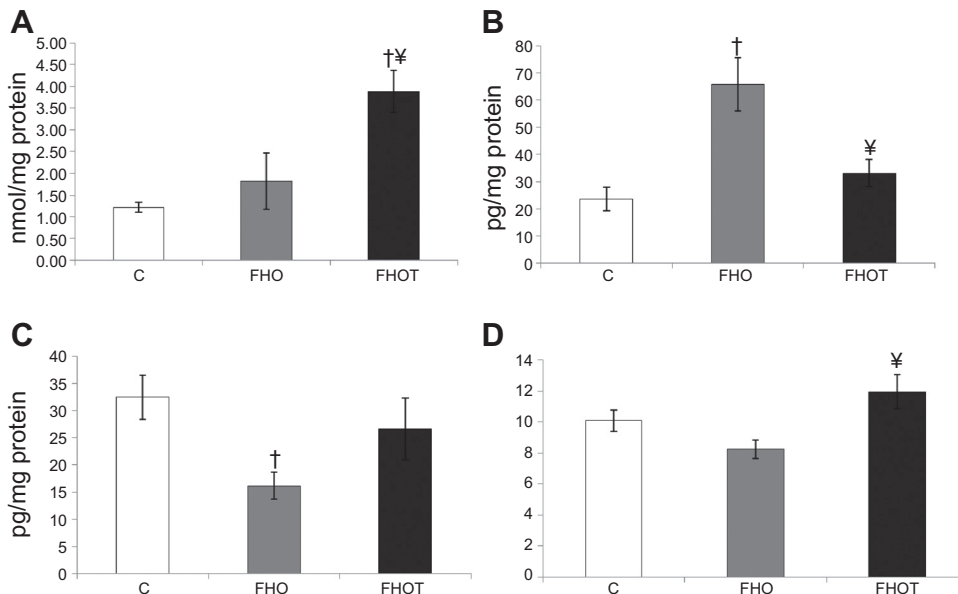

Fig. 2. *A*: nitric oxide (NO) metabolites (NOx); *B*: tumor necrosis factor-α (TNF-α); *C*: interleukin-10; *D*: GSH/GSSG in all studied groups. †$P < 0.05$ vs. C; ¥$P < 0.05$ vs. FHO.

Table 2. *Cardiac oxidative stress variables in studied groups*

	C	FHO	FHOT
Hydrogen peroxide, nmol/g tissue	4.90 ± 1.13	26.77 ± 8.61*	13.61 ± 2.58
Lipoperoxidation, cps/mg protein	2,666 ± 358	15,043 ± 1,333*	1,184 ± 357†
Protein oxidation, nmol/mg protein	3.00 ± 0.23	5.22 ± 0.67*	6.03 ± 0.44*
CAT, nmol/mg protein	0.84 ± 0.04	0.63 ± 0.05*	0.78 ± 0.05†
SOD, USOD/mg protein	16.21 ± 1.10	13.05 ± 0.86	26.82 ± 1.96*†
TRAP, μM of trolox	7.37 ± 1.19	10.72 ± 1.51	39.01 ± 7.00*†

Data are expressed as means ± SE. CAT, catalase; SOD, superoxide dismutase; TRAP, total antioxidant capacity. *$P < 0.05$ vs. C; †$P < 0.05$ vs. FHO.

groups. SOD activity was higher in the FHO group compared with C group and lower compared with the FHOT group (Table 3).

DISCUSSION

It is now well established that exercise training plays an important role in the prevention and/or treatment of several disorders. However, most studies have focused on aerobic exercise training, raising the question of to what extent resistance training alone or coupled with aerobic exercise would lower risk factors. International guidelines have recommended the practice of resistance exercise training along with aerobic exercise training (40, 49, 50). However, unlike aerobic training, the prescription of resistance training allows the modulation of several parameters, e.g., number of repetitions, number of sets, load intensity, and time interval between series (31). The many possible combinations of these variables may change the acute and chronic physiological effects of resistance training, making it difficult to use this type of exercise for at-risk populations. Thus the aim of this study was to evaluate the effects of combined exercise training in hypertensive ovariectomized rats undergoing fructose overload on cardiovascular autonomic modulation, inflammation, and oxidative parameters.

We observed an increase in physical capacity in the group that underwent combined exercise training, with higher values in both maximal aerobic and resistance tests. Another study from our group has demonstrated an improvement in physical capacity in ovariectomized rats after 8 wk of aerobic training (28). Similar findings were reported in premenopausal women (24), menopausal women not receiving hormone replacement therapy (2, 24), and menopausal women receiving hormone replacement therapy (24).

Furthermore, postmenopausal women usually show decreased exercise tolerance, muscle strength, and bone mass, together with increased body weight and prevalence of diabetes, osteoporosis, and cardiovascular diseases (47). The American College of Sports Sciences has recommended resistance exercises in addition to aerobic training for individuals with hypertension, peripheral vascular disease, Type 2 diabetes, obesity, and other conditions (50). In this context, resistance training promotes improvements in muscular strength and endurance and functional capacity, contributing to the overall health and quality of life and may also reduce risk factors for developing heart diseases, insulin resistance, and obesity, which are very common in Type 2 diabetic patients (40, 50). In our study, the animals undergoing combined exercise training showed greater strength gains compared with sedentary animals.

Regarding blood triglyceride levels, we observed an increase in the group undergoing fructose overload (FHO vs. C). The exposure of the liver to large amounts of fructose leads to stimulation of lipogenesis and a fast accumulation of triglycerides, which contributes to decrease insulin sensitivity (4). In fact, the FHO group showed insulin resistance, as demonstrated by decreased K_{itt} in relation to C group. These results are in line with the findings of other studies that use fructose overload in Wistar rats (8) and in ovariectomized SHR rats (44).

However, exercise training was able to normalize blood triglyceride levels, which may be related to the improved insulin sensitivity in the FHOT group. Sanches et al. (44) also observed a decrease in K_{itt} and triglyceride levels in ovariectomized SHR undergoing fructose overload after aerobic exercise training protocol. The improvement in insulin sensitivity after exercise training may be related to improved insulin signaling. In fact, some studies have demonstrated that exercise potentiates the effect of insulin on phosphorylation of IRS-2 with a consequent increase in the PI3 kinase activity (26). Furthermore, there is also a greater phosphorylation in serine of Akt, which is an essential protein that initiates the translocation of GLUT4 to the plasma membrane (51). Luciano et al. (34) have shown that exercise endurance in male rats adapted to pool improved insulin sensitivity by increasing the phosphorylation of IRS-1 and IRS-2, as well as the association of these proteins with PI3 kinase in insulin-stimulated animals, compared with control animals.

In the present study, fructose overload associated with ovarian hormone deprivation in SHR promoted an increase in both arterial pressure and HR and impairment in SAP variability parameters (VAR-SAP and LF-SAP) and in BRS compared with the C group. It should be stressed that the enhanced AP variability has been suggested to result from decreased BRS in hypertensive humans and animals (10, 35). Arterial baroreflex represents an important control system, buffering the magnitude of spontaneous arterial pressure fluctuations (36). Moreover, baroreceptors also exert tonic control over sympathetic activity (inhibition) and parasympathetic activity (stimulation). Thus impairment of the function of baroreceptors can play as permissive element to the establishment of primary changes in other mechanisms of cardiovascular control, failing to properly modulate the sympathetic and parasympathetic activity (27). In fact, experimental and clinical studies have demonstrated that dysautonomia (a dysfunction of the autonomic nervous system)

Table 3. *Renal oxidative stress variables in studied groups*

	C	FHO	FHOT
Lipoperoxidation, μmol/mg protein	1.69 ± 0.09	3.72 ± 0.19*	3.55 ± 0.13*
Protein oxidation. nmol/mg protein	1.97 ± 0.21	4.17 ± 0.36*	2.56 ± 0.12†
CAT, nmol/mg protein	1.72 ± 0.16	1.41 ± 0.09	2.03 ± 0.24
SOD.USOD/mg protein	9.48 ± 0.52	12.14 ± 0.74*	9.79 ± 0.25†

Data are expressed as means ± SE. *$P < 0.05$ vs. C; †$P < 0.05$ vs. FHO.

is present in a number of pathologies, e.g., hypertension, heart failure, diabetes mellitus, and other metabolic disorders (20, 42, 43, 55).

However, our results demonstrated that combined physical training promoted arterial pressure reduction associated with reduced VAR-SAP and LF-SAP (sympathetic vascular representatives), thus reflecting an improvement in the cardiovascular system. It should be noted that in a previous study of our group, using the same experimental model of menopause and hypertension undergoing fructose overload (but in the context of aerobic exercise training alone), we did not observe any reduction of arterial pressure (44). In this sense, the experimental results of the present study demonstrated an additional impact of combined exercise training on cardiovascular risk, since this type of training was able to reduce arterial pressure in this model of association of cardiometabolic dysfunctions after ovarian hormones deprivation.

With regard to inflammatory markers, the FHO group showed an increase in TNF-α and a reduction in IL-10 in the cardiac tissue compared with the C group. On the other hand, combined exercise training was effective to normalize IL-10 and reduce TNF-α in the cardiac tissue. Corroborating to our study, Renna et al. (41) have shown that chronic consumption of fructose in SHR rats induced damage to the cell membrane and increased TNF-α in the vessel. Since IL-10 acts as an antagonist of TNF-α by inhibiting the signaling of nuclear factor-κB (NF-κB) (19), we can hypothesize that combined exercise training was able to normalize IL-10, which resulted in a reduction of TNF-α, thus decreasing the pro-inflammatory profile in FHOT group in relation to FHO group.

Regarding NO bioavailability, studies in both humans and animals have shown that the shear stress induced by physical exercise is a potent stimulus for the release of vasorelaxing factors produced by the vascular endothelium, such as NO and endothelium-derived hyperpolarizing factor, thereby causing a reduction in BP values (30). Indeed, NO is a potent vasodilator and thus its role in BP control is extremely important. In this sense, studies have observed an increased NO bioavailability after moderate intensity physical training program, both clinically (11, 25) and experimentally (12). Claudino et al. (12) demonstrated that aerobic exercise training, performed on a treadmill adapted to rats for 8 wk, was able to promote an increase in NO in the cavernous body of male rats. Higashi et al. (25) observed an increase in NO accompanied by less reactive hyperemia in patients with essential hypertension who underwent an aerobic exercise training program. Corroborating these findings, the combined exercise training in the present study was also effective in improving the nitric oxide metabolism (NOx increase in FHOT vs. FHO group), suggesting increased NO bioavailability, which may have contributed to the reduction in AP in the FHOT group.

Regarding oxidative stress, the FHO group showed higher lipid peroxidation and protein oxidation (carbonyls) in the cardiac and renal tissues compared with the C group, together with an increase in hydrogen peroxidase in the cardiac tissue. Bertagnolli et al. (5) have shown increased lipid peroxidation in erythrocytes in aorta, as evaluated by CL, in male SHR compared with controls. A previous study has also observed a greater protein oxidation (carbonyls) in plasma of hypertensive rats (SHR) compared with normotensive Wistar rats (13).

On the other hand, animals undergoing combined exercise training (FHOT) decreased lipid peroxidation and hydrogen peroxide in the cardiac tissue and showed a decrease in protein oxidation in renal tissue compared with HFO group. Furthermore, the combined exercise training was able to promote an increase in cardiac SOD activity, showing an improvement in antioxidants, which may have contributed to the reduction of lipoperoxidation in this tissue. In fact, previous studies from our group have shown improvements in markers of oxidative stress in cardiac tissue, such as lipid peroxidation and antioxidant enzymes in old male Wistar rats (16) in ovariectomized female rats (28) and SHR (5) undergoing aerobic treadmill training.

Importantly, there is compelling evidence that the autonomic nervous system is involved in the genesis of cardiometabolic disorders, causing alterations in inflammatory status and oxidative stress (7, 15, 20, 48). In this sense, the data from this study suggest that the decrease in sympathetic modulation observed after combined exercise training may have positively influenced parameters of inflammation (reduced pro-inflammatory cytokine and increased anti-inflammatory), oxidative stress (reduction of ROS, lipid and protein oxidation, and increased antioxidant defenses) and NO bioavailability, by promoting metabolic and hemodynamic benefits in an experimental model of metabolic syndrome and menopause.

In conclusion, the results of this study demonstrate that fructose overload associated with hypertension and ovarian hormone deprivation promoted impairment in metabolic, cardiovascular, autonomic, inflammatory, and oxidative stress parameters, and combined exercise training was effective in reducing and/or normalizing such disorders in an experimental model.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: F.F.C., J.d.O.B., N.B., D.d.S.D., and C.M. performed experiments; F.F.C., J.d.O.B., N.B., D.d.S.D., C.M., S.F.L., M.-C.I., and K.D.A. analyzed data; F.F.C., J.d.O.B., N.B., D.d.S.D., C.M., S.F.L., M.-C.I., and K.D.A. interpreted results of experiments; F.F.C. prepared figures; F.F.C., M.M., M.-C.I., and K.D.A. drafted manuscript; M.M., M.-C.I., and K.D.A. conception and design of research; M.M., S.F.L., M.-C.I., and K.D.A. edited and revised manuscript; S.F.L., M.M., M.-C.I., and K.D.A. approved final version of manuscript.

REFERENCES

1. **Aebi H.** Catalase in vitro. *Methods Enzymol* 105: 121–126, 1984.
2. **Aiello EJ, Yasui Y, Tworoger SS, Ulrich CM, Irwin ML, Bowen D, Schwartz RS, Kumal C, Potter JD, McTiernan A.** Effect of yearlong, moderate-intensity exercise intervention on the occurrence and severity of menopause symptoms in postmenopausal women. *Menopause* 11: 382–388, 2004.
3. **Anderson ME.** Determination of glutathione and glutathione disulfide in biological samples. *Methods Enzymol* 13: 548–555, 1985.
4. **Basciano H, Federico L, Adeli K.** Fructose, insulin resistance and metabolic dyslipidemia. *Nutr Metab* 5: 1–14, 2005.

5. **Bertagnolli M, Campos C, Schenkel PC, De Oliveira VL, De Angelis K, Bello-Klein A, Rigatto K, Irigoyen MC.** Baroreflex sensitivity improvement is associated with decreased oxidative stress in trained spontaneously hypertensive rat. *J Hypertens* 24: 2437–2443, 2006.

6. **Bonora E, Moghettip Zancanaro C, Cigolini M, Querena M, Cacciatoni V, Corgnati A, Muggeo M.** Estimates of in vivo insulin action in man: comparison of insulin tolerance tests with euglycemic and hyperglycemic glucose clamp studies. *J Clin Endocrinol Metab* 68: 374–378, 1989.

7. **Borovikova LV, Ivanova S, Zhang M, Yang H, Botchkina GI, Watkins LR, Wang H, Abumrad N, Eaton JW, Tracey KJ.** Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature* 405: 458–462, 2000.

8. **Brito JO, Ponciano K, Figueroa D, Bernardes N, Sanches IC, Irigoyen MC, De Angelis K.** Parasympathetic dysfunction is associated with insulin resistance in fructose-fed female rats. *Braz J Med Biol Res* 41: 804–808, 2008.

9. **Buege JA, Aust SD.** Microsomal lipid peroxidation. *Methods Enzymol* 52: 302–10, 1978.

10. **Cheng Y, Kong XB, Su DF.** Effects of ketanserin on blood pressure variability in conscious spontaneously hypertensive rats. *Zhongguo Yao Li Xue Bao* 13: 235–238, 1992.

11. **Clarkson P, Montgomery H, Mullen M, Donald A, Powe A, Bull T.** Exercise training enhances endothelial function in young men. *J Am Coll Cardiol* 33: 1379–1385, 1999.

12. **Claudino MA, Priviero FBM, Teixeira C, De Nucci G, Antunes G, Zanesco A.** Improvement in relaxation response in corpus cavernosun from trained rats. *Urology* 63: 1004–1008, 2004.

13. **Condezo-Hoyos JL, Arribas SM, Abderrahim F, Somoza B, Gil-Ortega M, Diaz-Gil JJ.** Liver growth factor treatment reverses vascular and plasmatic oxidative stress in spontaneously hypertensive rats. *Hypertension* 30: 1185–1194, 2012.

14. **Cornelissen VA, Fagard RH, Coeckelberghs E, Vanhees L.** Impact of resistance training on blood pressure and other cardiovascular risk factors: a meta-analysis of randomized, controlled trials. *Hypertension* 58: 950–958, 2011.

15. **De Angelis K, Irigoyen MC, Morris M.** Diabetes and cardiovascular autonomic dysfunction: application of animal models. *Auton Neurosci* 28: 3–10, 2009.

16. **De Angelis KLD, Oliveira AR, Werner A, Bock P, Belló-Klein A, Irigoyen MC.** Exercise training in aging: hemodynamics, metabolic, and oxidative stress evaluations. *Hypertension* 30: 767–771, 1997.

17. **De Angelis K, Senador DD, Mostarda C, Irigoyen MC, Morris M.** Sympathetic overactivity precedes metabolic dysfunction in a fructose model of glucose intolerance in mice. *Am J Physiol Regul Integr Comp Physiol* 302: R950–R957, 2012.

18. **Evelson P, Travacio M, Repetto M, Escobar J, Llesuy S, Lissi EA.** Evaluation of total reactive antioxidant potential (TRAP) of tissue homogenates and their cytosols. *Arch Biochem Biophys* 388: 261–266, 2001.

19. **Fain JN, Madan AK, Hiler ML, Cheema P, Bahouth SW.** Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. *Endocrinology* 145: 2273–2282. Epub 2004.

20. **Farah V, Elased KM, Chen Y, Key M, Cunha T, Irigoyen MC, Morris M.** Nocturnal hypertension in mice consuming a high fructose diet. *Auton Neurosci* 130: 41–50, 2006.

21. **Flues K, Paulini J, Brito S, Sanches IC, Consolim-Colombo F, Irigoyen MC, De Angelis K.** Exercise training associated with estrogen therapy induced cardiovascular benefits after ovarian hormones deprivation. *Maturitas* 65: 267–271, 2010.

22. **Gonzalez FB, Llesuy S, Boveris A.** Hydroperoxide-initiated chemiluminescence: an assay for oxidative stress in biopsies of heart, liver, and muscle. *Free Radic Biol Med* 10: 93–100, 1991.

23. **Granger DL, Anstey NM, Miller WC, Weinberg JB.** Measuring nitric oxide production in human clinical studies. *Methods Enzymol* 301: 58–61, 1999.

24. **Green JS, Stanforth PR, Gagnon J, Leon AS, Rao DC, Skinner JS, Bouchard C, Rankinen T, Wilmore JH.** Menopause, estrogen, and training effects on exercise hemodynamics the HERITAGE study. *Med Sci Sports Exerc* 2: 74–82, 2002.

25. **Higashi Y, Sasaki S, Sasaki N, Nakagawa K, Ueda T, Yoshimizu A.** Daily aerobic exercise improves reactive hyperemia in patients with essential hypertension. *Hypertension* 33: 591–597, 1999.

26. **Howlett KF, Sakamoto K, Hirshman MF, Aschenbach WG, Dow M, White MF, Goodyear LJ.** Insulin signaling after exercise in insulin receptor substrate-2-deficient mice. *Diabetes* 51: 479–483, 2002.

27. **Irigoyen MC, Moreira ED, Ida F, Pires M, Cestari IA, Krieger EM.** Changes of renal sympathetic activity in acute and chronic conscious sinoaortic denervated rats. *Hypertension* 26: 1111–1116, 1995.

28. **Irigoyen MC, Paulini J, Flores LJF, Consolim-Colombo FM, Moreira ED, Belló-Klein A, De Angelis K.** Exercise training improves baroreflex sensitivity associated with oxidative stress reduction in ovariectomized rats. *Hypertension* 46: 998–1003, 2005.

29. **Kamide K, Rakugi H, Higaki J, Okamura A, Nagai M, Moriguchi K, Ohishi M, Satoh N, Tuck ML, Ogihara T.** The renin-angiotensin and adrenergic nervous system in cardiac hypertrophy in fructose-fed rats. *Am J Hypertens* 15: 66–71, 2002.

30. **Kingwell B, Sherrard B, Jennings G, Dart A.** Four weeks of cycle training increases basal production of nitric oxide from the forearm. *Am J Physiol Heart Circ Physiol* 272: H1070–H1077, 1997.

31. **Kraemer WJ, Ratamess NA.** Fundamentals of resistance training: progression and exercise prescription. *Med Sci Sports Exerc* 36: 674–688, 2004.

32. **Kuo TBJ, Lin T, Yang CCH, Li CL, Chen CF, Chou P.** Effect of aging on gender differences in neural control of heart rate. *Am J Physiol Heart Circ Physiol* 277: H2233–H2239, 1999.

33. **Laville M, Nazare JA.** Diabetes, insulin resistance and sugars. *Obes Rev* 10: 24–33, 2009.

34. **Luciano E, Carneiro EM, Carvalho CR, Carvalheira JB, Peres SB, Reis MA, Saad MJ, Boschero AC, Velloso LA.** Endurance training improves responsiveness to insulin and modulates insulin signal transduction through the phosphatidylinositol 3-kinase/Akt-1 pathway. *Eur J Endocrinol* 147: 149–157, 2002.

35. **Mancia G, Ferrari A, Gregorini L, Parati G, Pomidossi G, Bertinieri G, Grassi G, di Rienzo M, Pedotti A, Zanchetti A.** Blood pressure and heart rate variabilities in normotensive and hypertensive human beings. *Circ Res* 53: 96–104, 1983.

36. **Mancia G, Parati G, Pomidossi G, Casadei R, Di Rienzo M, Zanchetti A.** Arterial baroreflexes and blood pressure and heart rate variabilities in humans. *Hypertension* 8: 147–153, 1986.

37. **Marklund SL.** Superoxide dismutase isoenzymes in tissues and plasma from New Zealand black mice, nude mice and normal BALB/c mice. *Mutat Res* 148: 129–134, 1985.

38. **Moraes-Silva IC, Mostarda C, Moreira ED, Silva KA, dos Santos F, De Angelis K, Farah Vde M, Irigoyen MC.** Preventive role of exercise training in autonomic, hemodynamic, and metabolic parameters in rats under high risk of metabolic syndrome development. *J Appl Physiol* 116: 786–791, 2013.

39. **Pick E, Keisari Y.** A simple colorimetric method for the measurement of hydrogen peroxide produced by cells in culture. *J Immunol Methods* 38: 161–170, 1980.

40. **Pollock ML, Flanklin BA, Balady GJ, Chaitman BL, Fleg JL, Fletcher B, Limacher M, Pina IL, Stain RA, Williams M, Bazzarre T.** Resistance exercise in individuals with and without cardiovascular disease: benefits, rationale, safety, and prescription. *Circulation* 101: 828–833, 2000.

41. **Renna NF, Lembo C, Diez E, Miatello RM.** Role of renin-angiotensin system and oxidative stress on vascular inflammation in insulin resistence model. *International J Hypertens* 2013, doi:10.1155/2013/420979.

42. **Reznick AZ, Packer L.** Oxidative damage to proteins: spectrophotometric method for carbonyl assay. *Methods Enzymol* 233: 357–363, 1994.

43. **Rodrigues B, Figueroa DM, Mostarda CT, Heeren MV, Irigoyen MC, De Angelis K.** Maximal exercise test is a useful method for physical capacity and oxygen consumption determination in streptozotocin-diabetic rats. *Cardiovasc Diabetol* 6: 38, 2007.

44. **Sanches IC, Brito JO, Candido GO, Dias DS, Jorge L, Irigoyen MC, De Angelis K.** Cardiometabolic benefits of exercise training in an experimental model of metabolic syndrome and menopause. *Menopause* 19: 562–568, 2012.

45. **Sanches IC, Conti FF, Sartori M, Irigoyen MC, De Angelis K.** Standardization of a resistance exercise training: effects in diabetic ovariectomized rats. *Inter J Sports Med* 35: 323–329, 2014.

46. **Soares PP, Da Nobrega AC, Ushizima MR, Irigoyen MC.** Cholinergic stimulation with pyridostigmine increases heart rate variability and baroreflex sensitivity in rats. *Auton Neurosci* 30: 24–31, 2004.

47. **Sowers & La Pietra M MR.** Menopause: its epidemiology and potential association with chronic diseases. *Epidemiol Rev* 17: 287–302, 1995.

48. **Van Gaal LF, Mertens IL, De Block DE.** Mechanisms linking obesity with cardiovascular disease. *Nature* 444: 875–880, 2006.

49. **Williams AD, Almond J, Ahuja KD, Beard DC, Robertson IK, Ball MJ.** Cardiovascular and metabolic effects of community based resistance training in an older population. *J Sci Med Sport* 14: 331–337, 2011.

50. **Williams MA, Haskell WL, Ades PA, Amsterdam EA, Bittner V, Franklin BA, Gulanick M, Laing ST, Stewart KJ.** Resistance exercise in individuals with and without cardiovascular disease: 2007 update. *Circulation* 116: 572–584, 2007.

51. **Wojtaszewski JF, Higaki Y, Hirshman MF, Michael MD, Dufresne SD, Kahn CR, Goodyear LJ.** Exercise modulates post receptor insulin signaling and glucose transport in muscle-specific insulin receptor knock-out mice. *J Clin Invest* 104: 1257–1264, 1999.