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Exercise benefits the cardiac, autonomic and inflammatory responses to organophosphate toxicity

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ABSTRACT

The organophosphate, diisopropyl fluorophosphate (DFP), may impair cardiovascular, autonomic and immune function while exercise training is thougt to be restorative. Experiments determined effects of wheel exercise in C57B1 male mice, testing cardiovascular and autonomic function and characterization of the immunological profile. Sedentary (S) and exercise (ET) groups were treated with corticosterone (CORT) followed by injection of DFP. This model was associated with systolic and diastolic dysfunction in the S group, measured using echocardiography (ECHO). Chronic exercise ameliorated the cardiac deficit. Autonomic balance, accessed by heart rate variability (HRV), showed increased sympathetic and decreased parasympathetic modulation in S group. Autonomic balance in ET mice was not affected by DFP. Our DFP model resulted in mild neuroinflammation seen by increased IL5, IL12 and MIP2 in brain and plasma IL6 and IL1a. DFP had a negative impact on cardiac/autonomic function and inflammatory markers, effects reduced by exercise. Data suggest a beneficial effect of exercise training on the cardiovascular and autonomic responses to DFP/CORT.

1. BackGround

Exposure to environmental pollutants and chemical toxins is of great concern to global health. The detrimental effects occur throughout life, from conception to geriatric age. They influence subsequent generations via genomic and/or epigenetic adaptations. Incidence of disease associated with pesticide exposure have increased worldwide at alarming rates. This reflects the importance and urgency of addressing this environmental chemical crisis and the detection and identification of biological markers of exposure are also needed for the diagnosis and treatment of poisoning, in addition to occupational health monitoring for specific profiles of workers [1–8].

The most commonly used pesticides and herbicides fall into the chemical class of organophosphate (OP) which inhibit the cholinergic system via reversible and irreversible mechanisms [9]. At high doses, these toxic chemicals can be considered as warfare agents, forbidden by binding US and international agreements [10,11]. Nonetheless, infractions are widespread leading to OP exposures in various military and civilian cohorts. One well known example is the low-level chemical exposures which occurred during the 1991 Persian Gulf War [12,13]. There is also evidence of deliberate war- like exposure to sarin in the Japanese subway attacks [14] and more recently in the Syrian terrorist attacks [15,16].

In the USA, approximately 33 million pounds per year of OPs are used as agricultural chemicals [17]. Accidental or deliberate OP exposure is a major health concern of humans and animals [18]. Several reports have emphasized the association between high dose OP and the occurrence of developmental disorders [19–21]. Currently, there is a lack of available treatments capable of reversing harmful OP effects. Beyond that, little attention has been given to possible deleterious effects of low-dose, chronic exposure. Indeed, Kostoff et al. [22] showed that combinations of toxic stimuli can produce damage even when the

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exposure level of each member of the combination is less than the lowest exposure level of the member that produced damage when tested in isolation. Examples of chemical consequences are associated with organ damage including cardiac and immune dysfunction, autonomic imbalance and/or cardiomyopathies [9,4,23–25].

Previously, we demonstrated that low dose sarin exposure (GB; isopropyl methylphosphonofluoridate) caused autonomic imbalance leading to chronic cardiac and central nervous system abnormalities [24,26,27]. Abnormalities included ventricular dilatation, reduced contractility, altered electrophysiological and inotropic responses to beta adrenergic stimulation and potent long term, region specific effects on aminergic neurotransmitter systems [24,26,27].

Considering that there is little information on combined corticosterone (CORT) and OP CORT/DFP effects on cardiovascular and autonomic function, in the present study, we used a mouse model combining treatment with the stress hormone CORT and diisopropylfluorophosphate (DFP) [9,28,4]. This produces an acute neuro-immune cascade based on tests of cytokine molecular markers [9,4]. The present study focused on a combination approach, chemical exposure with a physical, non-invasive exercise training (ET) protocol (chronic wheel). Previous studies showed consistent and quantifiable health benefits of various types of exercise [29,30]. Recent studies from our group showed that moderate exercise Training (ET) improves exercise tolerance, cardiac function, autonomic modulation and oxidative and inflammatory profiles [31–33]. To date, there is little information on the effects of ET given prior to OP exposure. Our hypothesis is that an extended period of ET could prevent cardiac/autonomic dysfunction associated with acute administration of CORT and DFP.

2. Aim

The aim was to analyze the effects of previous aerobic exercise training on the cardiovascular, autonomic, and inflammatory profiles of animals exposed to DFP, an OP toxin and the stress hormone CORT.

3. Methods

C57BL male mice, 8 weeks old, were purchased from Jackson Laboratory (ME, USA). Mice were randomly divided into 2 groups: sedentary (S) and exercise trained (ET) (n = 8/group). They received water and food ad libitum and were housed (4/box) in a controlled environment with a photoperiod of 12:12-h light-dark and a temperature of 20 $^{\circ}$C. All procedures and protocols were approved by the ethics committee of the Veterans Affairs Hospital (VA, Miami, FL). Experiments were conducted in accordance with the guidelines of the National Institutes of Health document: Guide for the Care and Use of Laboratory Animals.

3.1. Exercise training protocol

Exercise training was performed using an electronic wheel at low intensity (20% of maximal running speed) for 1 h/day, 5 days/week for a 6-month period. All animals were adapted to the running procedure (10 min/day) for 1 week before beginning the ET protocol.

3.2. OP / CORT treatment

After 5 months of ET, the 2 groups were exposed to CORT and DFP. Mice received CORT in the drinking water (200 mg/L in 1.2% ethanol, EtOH) for 7 days. On day 8, DFP (Sigma-Aldrich, St. Louis, MO, USA) was diluted in saline (0.9% NaCl) and injected in a single dose (1.5 mg/kg s.c). Mice were killed by decapitation 1 month post-DFP.

3.3. Body fat measures using ECHO MRI (magnetic resonance imaging)

Body fat was measured at 5 and 6 months using ECHOMRI (Echo Medical Systems EchoMRI™, Houston, TX). For the test, a conscious mouse is placed in a clear cylindrical holder (4.7 cm diameter). The holder is placed in the MRI device and the animal is scanned. Three values were recorded and averaged for each parameter.

3.4. Heart rate variability (HRV)

The HRV was measured as described previously [34–36] pre (at 5 months of protocol) and post DFP exposure (6 Months of protocol).

3.5. Transthoracic echocardiogram (TTE)

TTE evaluations were performed according to guidelines of the American Society of Echocardiography using the same timeline as described for HRV analysis. Ultrasound data was acquired using a high-frequency ultrasound system (Vevo-1100 equipped with a MicroScan™ MS400 18–38 MHz cardiovascular transducer; Visual Sonics Inc., Toronto, Ontario, Canada). Mice were anesthetized with 4% isoflurane in pure oxygen (0.8 mL/min) using an induction chamber. Anesthesia was maintained using 1.5% isoflurane delivered by nose cone. Anesthetized mice were weighed, transferred to a heating pad, and placed in a supine position.

To determine the left ventricle (LV) structure and function, the parasternal long axis view (PLAX) was used to obtain images in 2D B-Mode with calculations of the following indices: end-systolic area (ESA), end-diastolic area (EDA), ejection fraction (EF), fractional shortening (FS) and cardiac output (CO). The M-mode short-axis view was obtained by turning the transducer scan head 90° from the PLAX. The short axis view at the level of the papillary muscles image in M-Mode was used to calculate end-diastolic diameter (LVEDD), end-systolic diameter (LVESD) and posterior wall thickness (PWT). Mitral inflow velocities were traced in apical view using pulse wave Doppler. This position was used to obtain the ejection time (ET), isovolumetric relaxation time (IVRT), isovolumetric contraction time (IVCT), maximal early diastolic peak velocity (E wave) and late peak velocity (A wave). Global cardiac function was evaluated using the myocardial performance index (MPI), which is the ratio of total time spent in isovolumetric activity to ejection time. Analysis was performed by a single observer using the VEVO 1100 System offline analysis module. Three values were recorded and averaged for each parameter from individual animals.

3.6. Dobutamine stress test

After baseline TTE records (2D B-Mode, 2 D M-Mode, Doppler and EKG) were made, mice were injected with dobutamine (DOBUT) (1 mg/kg, ip). The effect of DOBUT was visually and acoustically obvious in the Doppler tracings after 2–3 min. All ECHO measurements were repeated.

3.7. Brain dissection and plasma collection

Cytokines were measured in plasma and brain. Immediately after decapitation, whole brains were removed from the skull. Cerebral cortex was dissected with free hand on a cold glass plate (-85 °C). Blood from decapitation was collected in microtubes treated with anticoagulant. Red blood cells were separated by centrifugation and the supernatant designated plasma was removed and stored at -20 °C.

3.8. Cytokine analysis

Twelve cytokines (IFNγ, IL-1a, IL-1b, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, KC, MIP-2, TNFα,) were measured in mouse plasma and brain using the Quansys Imager and the Quansys reagents (Quansys Biosciences, Logan, Utah). Cytokines chemiluminescence assays were conducted at the Institute for Neuroimmune Medicine, Nova Southeastern

University, Fort Lauderdale, FL). Cytokines in brain and plasma (1-month post DFP) are expressed as pg/ml. Method details have been previously reported [37,38].

3.9. Statistical analysis

Statistical analysis of the data was performed using STATA software version 14.2. The data are presented as mean ± standard error. Two-way Repeated Measures Analysis of Variance (ANOVA) was used to calculate the DFP and DOBUT effect in the sedentary and exercise groups at different time points (pre and post DFP or at baseline and after DOBUT effect). A paired Student's *t*-test was used to compare the dependent variables before and after DFP exposure for sedentary and exercise groups. A *p-value* ≤ 0.05 was considered statistically significant.

4. Results

4.1. Body weight and body fat

At 5 months after first initiation of the protocol (Pre DFP), the S group presented higher body weight (BW) and body fat (BF) than the ET group. At the end of the protocol (1month post-DFP), the S and ET groups showed significant decreases in BW and BF compared to pre-DFP evaluation (Fig. 1).

4.2. Cardiovascular evaluation: TTE

TTE data (Table 1) showed that DFP altered cardiac function, but only in the S group. LV mass (LVM), end diastolic diameter (EDD) and end systolic diameter (ESD) were decreased in the S group as compared with the ET group. With regard to LV systolic function, the S group showed a decrease in ejection fraction (EF) and cardiac output (CO) and an increase in fractional shortening (FS). Additionally, the S group demonstrated a decrease in diastolic function, illustrated by an increased IVRT after DFP exposure. Furthermore, myocardial performance index (MPI), which is the ratio of total time spent in isovolumic activity (isovolumic contraction time and isovolumic relaxation time) to the ejection time, was higher in the S group when compared to ET group after DFP exposure (Fig. 2). This data documents the cardiovascular response to DFP along with the protective action of exercise.

4.3. Dobutamine stress test

TTE evaluation of LV systolic and diastolic function during the DOBUT stress test was used to test the hypothesis that drug induced increase in cardiac work induced by this drug may reveal a myocardial compromise. This change would not be manifest under resting conditions. We assessed the cardiac response to DOBUT at pre and post DFP exposure. Before DFP, the S and ET groups showed increased EF and FS and decreased EDD and ESD showing a positive response to DOBUT at baseline conditions (pre-DFP). However, when the test was performed after DFP the S group did not demonstrate an equivalent performance. In contrast, the ET group presented a positive inotropic response to DOBUT TTE after DFP, suggesting a positive effect of exercise in response to DOBUT (Fig. 3).

4.4. Autonomic Evaluation: HRV

Fig. 4 shows a significant increase in LF/HF ratio in the S group exposed to DFP ($p = 0.048$), not observed in the ET group ($p = 0.239$). These changes are related to a significant decrease in parasympathetic modulation (HF%) after DFP in the S group ($p = 0.038$), which did not occur in the ET group ($p = 0.199$) (Fig. 4). Sympathetic modulation (LF %) was increased (not statistically significant, $p = 0.076$) in the S group and did not change in the ET group with DFP ($p = 0.608$) (Fig. 4).

4.5. Immunological evaluation: cytokines

Chemiluminescent assays of an array of cytokines (IFNγ, IL-1a, IL-1b, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, TNFα) and chemokines (KC, MIP-2) known to be involved in the neuro-inflammatory response in plasma and brain were measured at 1 month post-DFP exposure. DFP increased expression of IL5, IL12 and MIP2 in cerebral cortex (Table 2). Moreover, the ET group showed increase in brain anti-inflammatory cytokine, IL10. In plasma, acute non-lethal exposure to nerve agent surrogate, DFP, increased expression of IL6 and IL1a (Table 2).

5. Discussion

The objective of this study was to determine the effects of DFP + CORT exposure on cardiovascular function, autonomic balance and the immunological profile of mice submitted to long term aerobic exercise training. We observed that ET was able to prevent inflammatory and cardio/autonomic dysfunction promoted by treatment

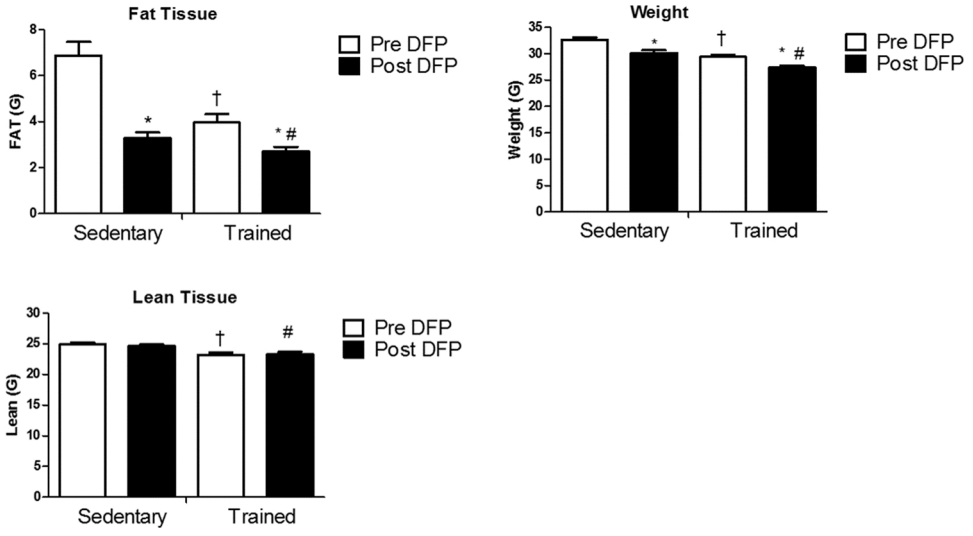

Fig. 1. Body weight and body Fat Measurements in Sedentary (n = 8) and trained (n = 8) groups. *p < 0.05 vs pre DFP in the same group; † vs sedentary pre DFP; # vs sedentary post DFP.

Table 1
ECHO Parameters - All values are expressed as the mean ± SE analyzed using 2-way ANOVA and Bonferoni post hoc test.

Parameter	Sedentary Group				Active Group				Sedentary Group		Active Group	
	Before DFP		After DFP		Before DFP		After DFP					
	Pre DOB	Post DOB	Pre DOB	Post DOB	Pre DOB	Post DOB	Pre DOB	Post DOB	DFP	DOB	DFP	DOB
EDA (mm^2)	23.04 ± 0.76	18.51 ± 1.17	19.98 ± 0.78	19.55 ± 0.77	21.57 ± 0.46	19.80 ± 1.13	20.59 ± 0.70	20.08 ± 0.55	F(1,31) = 1.09 **P = 0.3045**	F(1,31) = 7.25 **P = 0.0113**	F(1,27) = 0.20 **P = 0.6560**	F(1,27) = 2.32 **P = 0.1391**
ESA (mm^2)	8.38 ± 0.61	5.63 ± 0.47	8.39 ± 0.68	7.38 ± 0.58	8.39 ± 0.50	5.21 ± 0.43	6.13 ± 0.65	5.66 ± 0.46	F(1,31) = 2.13 **P = 0.1541**	F(1,31) = 10.28 **P = 0.0031**	F(1,27) = 2.49 **P = 0.1260**	F(1,27) = 11.22 **P = 0.0024**
FS (%)	40.74 ± 2.90	47.93 ± 3.25	45.67 ± 2.67	52.05 ± 5.01	34.47 ± 2.09	49.16 ± 3.24	39.61 ± 4.09	53.95 ± 3.65	F(1,29) = 1.59 **P = 0.2175**	F(1,29) = 3.64 **P = 0.0665**	F(1,27) = 2.36 **P = 0.1358**	F(1,27) = 20.34 **P = 0.0001**
EF (%)	82.35 ± 1.56	86.28 ± 1.10	77.17 ± 1.36	80.82 ± 1.82	80.69 ± 1.76	89.19 ± 1.54	83.06 ± 1.72	87.54 ± 1.48	F(1,29) = 12.77 **P = 0.0013**	F(1,29) = 6.57 **P = 0.0158**	F(1,25) = 0.01 **P = 0.9244**	F(1,25) = 16.41 **P = 0.0004**
HR (bpm)	453.7 ± 7.42	477.4 ± 7.95	485.7 ± 9.90	466.5 ± 12.78	438.6 ± 6.83	477.8 ± 11.67	432.1 ± 15.46	443 ± 17.80	F(1,30) = 0.93 **P = 0.3423**	F(1,30) = 0.08 **P = 0.7845**	F(1,27) = 2.45 **P = 0.1288**	F(1,27) = 3.90 **P = 0.0587**
EDD (cm)	0.379 ± 0.009	0.280 ± 0.014	0.320 ± 0.011	0.300 ± 0.017	0.370 ± 0.009	0.319 ± 0.017	0.357 ± 0.011	0.3039 ± 0.012	F(1,30) = 1.36 **P = 0.2522**	F(1,30) = 17.09 **P = 0.0003**	F(1,27) = 1.13 **P = 0.2980**	F(1,27) = 16.45 **P = 0.0004**
ESD (cm)	0.224 ± 0.009	0.146 ± 0.014	0.175 ± 0.013	0.147 ± 0.021	0.243 ± 0.011	0.161 ± 0.012	0.216 ± 0.017	0.139 ± 0.011	F(1,30) = 2.15 **P = 0.1533**	F(1,30) = 12.02 **P = 0.0016**	F(1,27) = 3.58 **P = 0.0694**	F(1,27) = 38.12 **P = 0.0000**
RWT	0.611 ± 0.072	0.987 ± 0.099	0.650 ± 0.044	0.862 ± 0.100	0.564 ± 0.046	0.861 ± 0.143	0.675 ± 0.104	0.809 ± 0.100	F(1,30) = 0.29 **P = 0.5969**	F(1,30) = 12.54 **P = 0.0013**	F(1,27) = 0.08 **P = 0.7808**	F(1,27) = 4.48 **P = 0.0436**
ET (ms)	45.31 ± 1.45	42.04 ± 1.69	44.39 ± 1.21	42.79 ± 1.40	47.26 ± 2.44	43.17 ± 1.53	49.03 ± 2.30	46.84 ± 1.51	F(1,30) = 0.01 **P = 0.9395**	F(1,30) = 2.94 **P = 0.0970**	F(1,25) = 1.92 **P = 0.1779**	F(1,25) = 2.56 **P = 0.1223**
IVRT (ms)	15.75 ± 0.492	16.39 ± 0.307	18.20 ± 0.905	18.64 ± 0.747	16.62 ± 0.355	15.15 ± 0.435	15.59 ± 0.743	15.85 ± 0.728	F(1,30) = 14.44 **P = 0.0007**	F(1,30) = 0.79 **P = 0.3816**	F(1,22) = 0.11 **P = 0.7466**	F(1,22) = 1.17 **P = 0.2916**
CO (ml/min)	17.71 ± 0.965	9.161 ± 1.318	13.290 ± 1.093	11.277 ± 1.597	15.956 ± 1.127	14.155 ± 2.208	15.552 ± 2.008	11.498 ± 1.615	F(1,28) = 0.35 **P = 0.5595**	F(1,28) = 14.08 **P = 0.0008**	F(1,27) = 0.75 **P = 0.3954**	F(1,27) = 2.60 **P = 0.1183**
MPI	0.350 ± 0.015	0.393 ± 0.011	0.419 ± 0.022	0.438 ± 0.023	0.357 ± 0.019	0.345 ± 0.014	0.323 ± 0.027	0.352 ± 0.024	F(1,29) = 10.16 **P = 0.0034**	F(1,29) = 3.33 **P = 0.0783**	F(1,23) = 0.37 **P = 0.5498**	F(1,23) = 0.17 **P = 0.6860**
E/A ratio	1.23 ± 0.04	1.47 ± 0.083	1.34 ± 0.053	1.27 ± 0.039	1.16 ± 0.007	1.28 ± 0.066	1.50 ± 0.058	1.46 ± 0.094	F(1,15) = 0.01 **P = 0.9344**	F(1,15) = 2.30 **P = 0.1499**	F(1,15) = 18.63 **P = 0.0006**	F(1,15) = 0.40 **P = 0.5370**

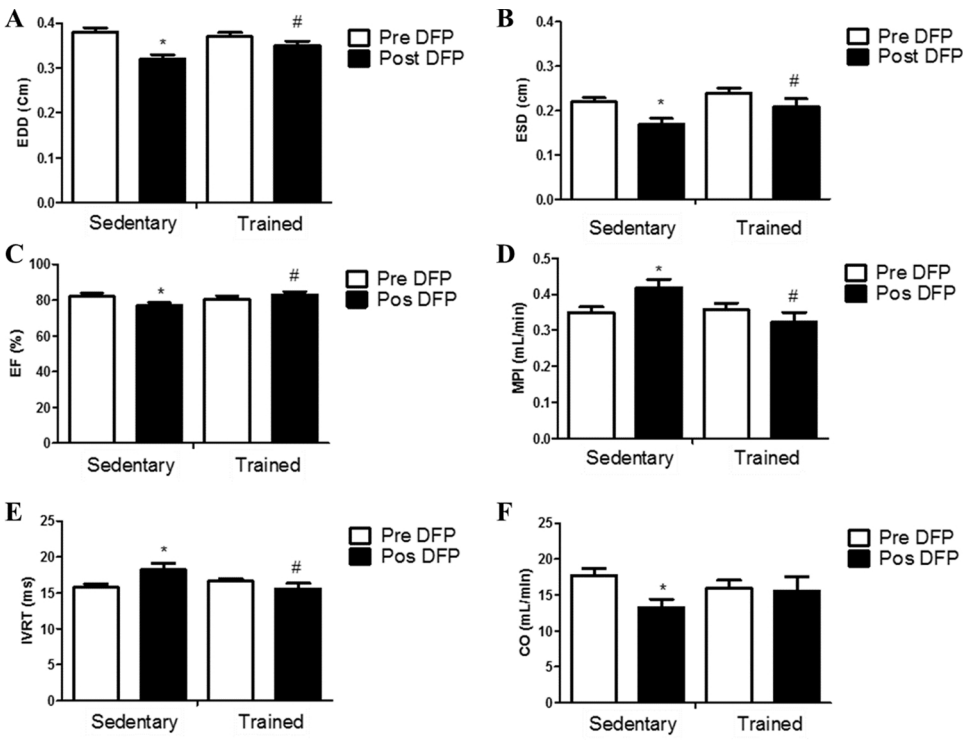

Fig. 2. Cardiac function in Sedentary (n = 8) and trained (n = 8) groups. **(A)** End diastolic diameter (EDD); **(B)** End Systolic diameter (ESD); **(C)** Ejection Fraction (EF); **(D)** myocardial performance index (MPI); **(E)** isovolumetric relaxation time (IVRT); **(F)** cardiac output (CO). *p < 0.05 vs. pre DFP in the same group, #p < 0.05 vs sedentary post DFP, ¥ p < 0.05 vs sedentary pre DFP.

with DFP + CORT. Our findings provide a rationale for the development of an experimental model for the study of the beneficial effects of exercise paradigms on the cardiovascular-immune outcomes in the toxicity of common use as chemical agents. Domestic use of OPs has long been prohibited. Even so, OPs remain in mainstream use because of their need for use in agriculture and insect control. They are widely used to reduce crop loss and increased harvest productivity. [39].

The mechanisms by which OP compounds produce brain and heart toxicity is still not clear. It is likely that the toxins act via the cholinergic systems (autonomic nervous system (ANS)), resulting in accumulation

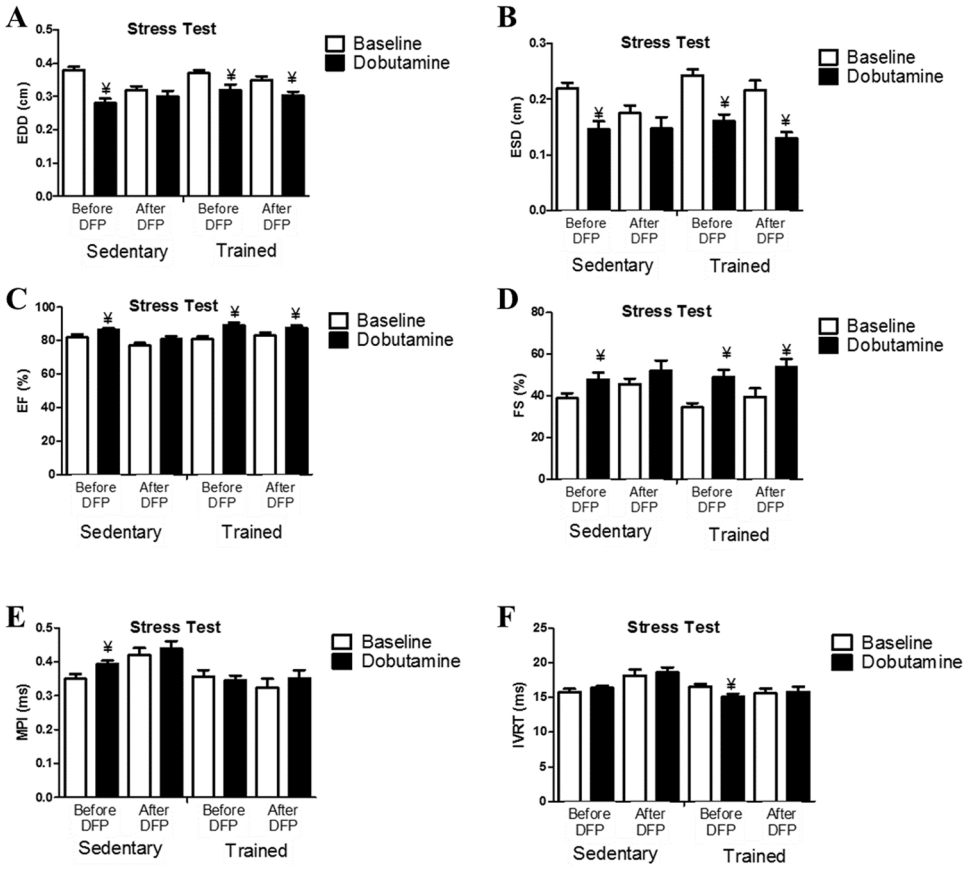

Fig. 3. Dobutamine stress test before and after DFP in Sedentary (n = 8) and trained (n = 8) groups. **(A)** End diastolic diameter (EDD); **(B)** End Systolic diameter (ESD); **(C)** Ejection Fraction (EF); **(D)** Fraction shorting (FS); **(E)** isovolumetric relaxation time; **(F)** myocardial performance index (MPI). ¥ p < 0.05 vs. pre dobutamine same group.

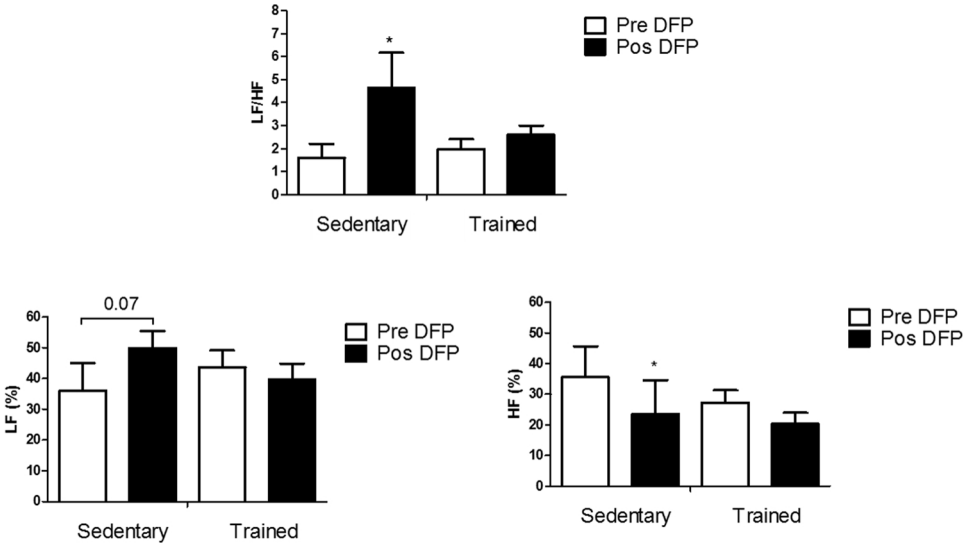

Fig. 4. LF/HF (Low Frequency/High Frequency ratio) in the sedentary and trained group before and after DFP exposure. LF abs (Low Frequency absolute value) representing the sympathetic modulation before and after DFP exposure in the sedentary and trained groups. HF abs (High Frequency absolute value) representing the parasympathetic modulation before and after DFP exposure in the sedentary and trained groups. The data are presented in mean ± standard error of the mean. * = p ≤ 0.05 when compared pre and post DFP.

Table 2
Plasma and brain cytokines in sedentary and trained groups. Data are presented in mean ± standard error of the mean. * = p ≤ 0.05 when compared sedentary group vs trained group.

Tissue	PLASMA			BRAIN		
Cytokines (pg/ml)	Sedentary	Trained	P-Value	Sedentary	Trained	P-Value
IFNy	2.03 ± 0.63	4.40 ± 1.72	0.1965	12.34 ± 1.69	9.11 ± 1.43	0.1769
IL1a	1.09 ± 0.17	3.43 ± 1.11	0.0210*	6.20 ± 1.34	5.03 ± 0.33	0.4418
IL1b	13.62 ± 3.63	24.35 ± 8.84	0.2601	59.24 ± 9.05	70.63 ± 11.46	0.4510
IL2	6.65 ± 1.82	6.21 ± 1.53	0.8571	13.17 ± 2.95	15.31 ± 2.61	0.5972
IL4	1.81 ± 0.86	1.38 ± 0.67	0.7070	7.23 ± 2.69	12.08 ± 3.02	0.2512
IL5	16.87 ± 4.79	13.18 ± 1.95	0.5110	26.65 ± 2.99	40.13 ± 4.56	0.0252*
IL6	13.90 ± 3.66	3.96 ± 1.71	0.0395*	10.80 ± 2.06	11.89 ± 2.70	0.7514
IL10	6.05 ± 1.60	5.03 ± 0.69	0.5883	17.68 ± 0.55	25.52 ± 4.00	0.0291*
IL12	12.23 ± 3.20	9.20 ± 3.23	0.5201	20.78 ± 1.68	32.01 ± 5.61	0.0316*
KCCXCL1	169.79 ± 20.29	160.69 ± 22.05	0.7702	48.41 ± 7.95	34.08 ± 9.65	0.2685
MIP2	19.44 ± 3.53	18.02 ± 3.91	0.7921	34.40 ± 2.98	49.84 ± 8.52	0.0471*
TNFa	1.36 ± 0.41	2.21 ± 0.58	0.2498	3.96 ± 0.55	5.11 ± 0.76	0.2391

of acetylcholine due to AChE inhibition. These chemicals might also cause an inflammatory response, leading to chemically-induced myocarditis and risk for cardiac complications with heart failure and arrhythmias. Indeed, animals exposed to pesticides present electrocardiogram abnormalities, impaired systolic and diastolic performance, functional remodeling and histopathological findings, such as haemorrhage, vacuolisation, signs of apoptosis and degeneration as well as increased the oxidative stress and oxidative modifications in the genomic DNA content of the cardiac tissues [40,41].

TTE analysis showed that DFP decreases FS, EF and CO, suggesting a negative chronotropic and inotropic effects in the heart. Similarly, the decrease in ESD after DFP is likely due to decreased left ventricular emptying associated with depressed myocardial function. The effect of decreasing ESD and EDD may also be explained by an elevation in preload on end diastolic volume [42]. Several studies suggest that OPs may act by binding directly to M2 muscarinic receptors, producing changes in cardiac function [43]. Discrepancies in cardiovascular and autonomic effects of OP exposure can be attributed to factors such as chemical dose, OP structure, anesthesia and environmental conditions.

The animals were also evaluated under a pharmacological stressor, (DOBUT), which has been used for the diagnosis of heart disease. The sedentary group (S) showed a reduction on EDD, ESD, EF as well as an increase in FS when challenged with dobutamine, suggesting a deficiency on the cardiac demand.

On the other hand, in the animals submitted to exercise regime, the magnitude of the dobutamine stress response remained unchanged. In terms of clinical testing, data based on a log-rank test using dobutamine stress, demonstrated that patients following an exercise routine had a lower probability of cardiac events during follow-up than sedentary patients ($p < 0.001$) [44]. Tsarouhas et al. [45] showed that, in patients with reduced EF, moderate, unsupervised, everyday physical activity ameliorated the lipid and glycemic profile, with simultaneous attenuation of inflammation and oxidative stress.

With regard to autonomic control, this was evaluated by the HRV analysis. This method is used to assess the ANS (autonomic nervous system) in various populations [46]. The development and implementation of a standardized protocol for HRV analysis in combination with cardiac TTE has the potential to add a dimension for preventive and remedial approaches to the fields of neurotoxicity and cardiovascular disease [47,48].

Acute OP poisoning causes death primarily by overstimulation of muscarinic and nicotinic receptors leading to suffocation due to bronchospasm, bronchorrea, respiratory muscle paralysis, and respiratory depression which can be accompanied by convulsions and coma (Goodman and Gilman's The Pharmacological Basis of Therapeutics). Chronic sublethal exposures cause autonomic imbalance which are associated with cardiomyopathy and impaired inotropic responsivity to beta-adrenergic stimulation [24]. In the present study, the S group demonstrated a higher sympathetic modulation and increased LF/HF ratio after DFP exposure. It was observed that exercise training was effective in preventing the increase of the sympathetic modulation as well as the increase of the LF/HF ratio after DFP. A reduced

sympathetic modulation decreases the amount of work and oxygen consumed by the heart via a reduction in resting heart rate and myocardial contractility [49]. An increased vagal modulation directly acts on the sinus node and the myocardium and hinders sympathetic influences [49–51]. It is important to note that a decreased HRV is associated with a bad prognosis for cardiovascular/metabolic disorders. Studies show that a reduction in HRV is established as an independent risk factor for arrhythmia and general mortality in patients following acute myocardial infarction [52]. This reduction is related to adrenergic hyperactivity and decreased cardiac parasympathetic activity [53]. Therefore, physical training may modulate the decline of function associated with DFP exposure, preventing abnormalities in autonomic control and reducing the risk of cardiovascular morbidity and mortality.

One of the mechanisms for which the exercise may modulate the autonomic balance is by suppressing angiotensin II expression. Angiotensin II is known to inhibit vagal cardiac activity [49,54]. Nitric Oxide (NO) may also play a role in increasing cardiac vagal control and, in doing so, may indirectly inhibit sympathetic influences [55]. Exercise training has been found to improve endothelial function [56] and NO bioavailability [57]. Angiotensin II and NO have been proposed as potential mediators in this relationship. However, more research is required to substantiate these claims, particularly with respect to NO [58].

Neuroinflammation is associated with enhanced expression of pro-inflammatory cytokines and chemokines (e.g., TNF-α, IL-1, IL-6, CCL-2) [59,60]. Toxic chemical exposures that enhance the level or duration of pro-inflammatory responses will consequently lead to more severe pathologies. Detrimental effects could include dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis and changes within the immune signaling network [61]. While there is much information on the neurological and immunological effects of OPs, it is unclear how chemical exposure may affect the activation of the peripheral inflammation in a chronic model of exposure to DFP [4]. Our data indicate increased level of IL-1a after the acute non-lethal exposure to DFP, demonstrating cellular system reactivity to stress in the periphery. Moreover, we found a significant decrease of IL-6, a cytokine with dual modulatory effect. Lower levels of IL-6 maintain a basal immune response stimulation while reduce IL-6 competence as an anti-inflammatory mediator by minimizing its direct inhibitory effect on TNF-alpha (an important mediator of the transcription of pro-inflammatory molecules) and IL-1a (a pro- inflammatory cytokine). Additionally, IL-6 efficacy is also limited indirectly by the reduced activation of other molecules, such as IL-10 (the cytokine synthesis inhibitory factor) or the IL-1 receptor antagonist (IL1ra). The equilibrium on the modulation of the peripheral inflammation and its dual activity can be explained by the effect of DFP on the interplay between stress and proper function of the autonomic nervous axes.

Previously, O'Callaghan et al. [4] classified their model as a neuro immune-based disorder, however the long-term model as presented in our study shows only mild inflammation indicated by a limited increase of brain levels of IL-5, IL-12 and MIP2. Although the brain inflammatory response seems to be moderate at this point, we can still observe a persistent pro-inflammatory environment promoted by IL-5. This is assisted by increased levels of IL-12 and MIP2. This assures the persistence of the immune reactivity modulated by events such as the IL4-mediated suppression of IFNγ that favors adaptive immunity chemoattraction, cell migration and macrophage activation. The ET group showed an increased level of IL-10 in the CNS. The presence of this anti-inflammatory cytokine indicates some level of containment of inflammation or inflammatory resolution in the brain. This highlights the separation between short and long-term OP exposure as well as central vs peripheral systems. Previous work suggests that physical activity has anti-inflammatory effects and additionally reduces blood viscosity while reduces thrombotic tendencies [62]. These effects may be biologically linked with the cholinergic anti-inflammatory pathway acting to enable the immune and autonomic systems [63].

6. Conclusion

Experimental exposure to DFP provides an animal model which recapitulates the effects of chronic toxicity of the OPs. It is relevant to military, ocupational or high risk environmental conditions. Our study showed that long-term low intensity exercise decreased sympathetic activity and preserved vital cardiac and inflammatory parameters in DFP treated mice. These findings support the use of experimental models of toxicity to study the long term effects of aerobic exercise/conditioning on the autonomic and cardiovascular changes triggered by OP exposures. Experiments focused on the modulatory role of a simple wheel-based exercise program to study chemical toxicity will contribute to a better understanding of the effects of OPs frequently used as a neurotoxic agent.

Disclosure Statement

The authors indicate that they do not have any conflicts of interest. No competing financial interests exist.

Transparency document

The Transparency document associated with this article can be found in the online version.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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