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Original article

Therapeutic and preventive effects of exercise on cardiometabolic parameters in aging and obese rats

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SUMMARY

Background and aims: Aging, obesity and sedentarism are among the most important predictors of cardiometabolic diseases. Aiming to reduce the impact of the combination of these three factors, we tested the therapeutic and preventive effects of exercise in aging and obese rats on the following cardiometabolic disease risk parameters: body fat, blood pressure, blood lipids, and glycemic homeostasis.
Methods: Eighteen male Wistar rats (initial age = 4 months, and final age = 14 months) were randomly distributed into three aging and obese groups: sedentary, therapeutic exercise and preventive exercise. Food and caloric intake, body adiposity, muscle mass, cardiovascular parameters, biochemical markers, glycemic homeostasis, and gene expression of insulin-dependent, insulin-independent and insulin resistance pathways in skeletal muscle were evaluated.
Results: Therapeutic and preventive exercises were associated with higher food and caloric intake, and expression of TBC1D1 in the soleus muscle, as well as lower total cholesterol/HDL and LDL/HDL ratios, glucose levels at the end (90 min) of the glucose tolerance test and IKBKB expression in the gastrocnemius and soleus muscles. Only the preventive exercise improved the cardiovascular and body composition parameters, glucose tolerance, insulin resistance and insulin sensitivity, besides reducing total cholesterol, triglycerides, triglycerides/HDL ratio, plasmatic insulin and MAPK8 expression in soleus. The preventive exercise group also presented greater expression of INRS, IRS1, IRS2, PIK3CA, AKT1, and SLC2A4 in gastrocnemius and soleus, TBC1D1 in gastrocnemius, and AKT2 and PRKAA1 in soleus.
Conclusions: Therapeutic exercise promoted some improvements on cardiometabolic parameters in aging and obese rats, however, the best benefits were achieved through the preventive exercise.

1. Introduction

The association of aging, obesity and sedentary lifestyle is critical for the development of cardiometabolic diseases [1]. Aging promotes progressive structural and functional changes that precipitate the development of several pathological processes, including hypertension, dyslipidemias and type 2 diabetes [2]. Changes that leads to these dysfunctions include reduced protein synthesis and decreased level of physical activity, culminating in decline in aerobic fitness and increase in adipose tissue deposits [3].

Even though of obesity is a multifactorial cause condition involving biological, genetic and environmental factors, the key elements are excessive intake of energy-dense foods and low physical activity [1,4]. Although mechanisms linking diet-induced

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Abbreviations

ACTB	actin, beta
AKT1	v-akt murine thymoma viral oncogene homolog 1
AMPK	protein kinase, AMP-activated
ANOVA	analysis of variance
AUC	area under the curve
BAT	brown adipose tissue
cDNA	complementary DNA
EAT	epididymal adipose tissue
ELISA	enzyme-linked immunosorbent assay
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GDC	genomic DNA control
GLUT4	glucose transporter type 4
GTT	glucose tolerance test
HOMA-β	Homeostatic Model Assessment – β cell
HOMA-IR	Homeostatic Model Assessment – Insulin Resistance
IKBKB	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta
IL-6	interleukin-6
IMS/UFBA	Instituto Multidisciplinar em Saúde at the Universidade Federal da Bahia
INSR	insulin receptor
IRS1	insulin receptor substrate 1
JNK	N-terminal c-Jun kinase
MAP	mean arterial pressure
MAPK8	mitogen-activated protein kinase 8
MAT	mesenteric adipose tissue
NEFA	non-esterified fatty acids
PCR	polymerase chain reaction
PPC	positive PCR control
PRKAA1	protein kinase, AMP-activated, alpha 1 catalytic subunit
qPCR	real time quantitative polymerase chain reaction
QUICKI	Quantitative Insulin Sensitivity Check Index
RAT	retroperitoneal adipose tissue
RTC	reverse transcription control
SAT	subcutaneous adipose tissue
SLC2A4	solute carrier family 2, member 4
TNF-α	tumor necrosis factor alpha
VAT	visceral adipose tissue

obesity to its associated cardiometabolic diseases have not been fully elucidated, it appears that inflammation plays a central role in this relationship [4]. Evidence suggests that obesity leads to a proinflammatory stage of metabolic cells (adipocytes, hepatocytes, and myocytes) that results in increased secretion of proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) [4–7]. The release of these and other cytokines, together with elevated levels of non-esterified fatty acids (NEFA), gradually leads to obesity-induced inflammation, promoting cardiometabolic dysfunctions such as insulin resistance, progressing to type 2 diabetes and coronary artery disease [4,8,9].

Meanwhile, a growing body of evidence indicates that physical exercise has therapeutic potential for improving cardiometabolic health even among those who are obese. It has been demonstrated in rodents and humans that exercise has contributed to improved body composition and inflammation and increases insulin sensitivity [10–12]. A recent study with young obese rats demonstrated that exercise was able to reduce cardiometabolic risk even when these treatments were implemented simultaneously with the administration of high-fat diet [13]. However, exercise efficacy has not been adequately tested under mutual experimental conditions of aging and diet-induced obesity. In addition, to date we have not found a study investigating the impact of lifelong physical exercise (i.e., preventive exercise) on cardiometabolic status during aging in an experimental condition designed to promote obesity. Thus, the purpose of this study was to evaluate the therapeutic and preventive effects of exercise on cardiometabolic disease risk parameters including body fat, blood pressure, blood lipids, and glycemic disorders induced by aging and obesity. We tested the hypotheses that (i) exercise could attenuate cardiometabolic dysfunctions resulting from the combination of aging and diet-induced obesity and (ii) preventive exercise (compared to therapeutic exercise) would result in better cardiometabolic outcomes.

2. Materials and methods

2.1. Animals and experimental design

Eighteen male Wistar rats with treadmill exercise aptitude were used, initially, at four months old (~18 weeks), from the Animal Breeding Center of the Instituto Multidisciplinar em Saúde at the Universidade Federal da Bahia (IMS/UFBA). The animals were housed in polypropylene boxes (four rats per box) with light control (12 h of light, 7 a.m. to 7 p.m.) at 21 ± 2 °C and free access to water and feed. Throughout the experiment the animals received commercial standard (Presence, Brazil) or casein-based purified high-fat feeds (Pragsoluções Biociências, Brazil). The bromatological composition of diets used were described in Table 1 and more detailed information about the diets can be seen in supplementary material 1 and 2.

The selection of animal runners took place during a seven day period of ambientation and acclimation to training apparatus. All animals were placed for two consecutive days in a motorized electric treadmill with six stalls (AVS Projetos, Brazil) disconnected for 5 min for ambientation. After this period, for five consecutive days, the treadmill was connected and speed gradually increased until reaching 10 m/min. At this speed, the animals ran for five minutes at 10% inclination. When necessary, mechanical stimuli were fired to motivate the animals to run. At the end of seven days, only one (~5%) animal showed an inconsistent running pattern, and therefore was considered unfit and excluded from the study.

The eighteen selected rats at four months of age were randomly divided into two groups: a group that was kept sedentary (n = 12) and a group that was subjected to preventive aerobic exercise (n = 6). At the age of nine months, both groups started to receive a high-fat diet for obesity induction. When they reached 12 months old, the sedentary group was randomly

Table 1
Bromatological composition of the experimental diets.

Item	Experimental diet	
	Standard diet	High-fat diet
Dry matter (%)	87.0	85.1
Ashes (%)	10.0	0.8
Crude protein (%)	23.0	15.1
Ethereal extract (%)	4.0	30.5
Total Carbohydrates (%)	62.0	42.8
Metabolizable energy (Mcal/kg)	2.5	5.1

subdivided into two groups (n = 6/group), which were or were not subjected to therapeutic aerobic exercise for two months. Therefore, the study consisted of three aging and obese experimental groups (Fig. 1): sedentary, therapeutic exercise and preventive exercise.

2.2. Evaluation of the aerobic capacity

Before and during (every six weeks) the training protocol, the groups were submitted to maximum aerobic capacity tests, as previously described by other authors [14]. The test was performed until exhaustion of the animals, and the fatigue criterion used was the inability to maintain the running pattern. The test results were used to determine and adjust the intensity of the exercise.

2.3. Training protocol (moderate intensity aerobic exercise)

Intensity, frequency and duration parameters for establishing the training protocol were based on international guidelines for the prescription of cardiorespiratory endurance exercise [15], as follows:

- Modality: aerobic exercise in electric treadmill with 10% inclination;
- Intensity: 60% of maximal aerobic capacity (moderate);
- Time: 60 continuous minutes;
- Frequency: alternate days.

During the first ten training sessions, the intensity and duration were progressively increased until reaching the target volume, which was maintained until the end of the experiment (see details in supplementary material 3). During the experiment, the groups that were kept sedentary did acclimation activity (five minutes at a velocity of 10 m/min and 10% inclination) three times a week, so they did not lose aptitude for exercise during the aging. All groups were trained in the afternoon.

2.4. Body weight and cardiovascular parameters

Animals' body weight was measured using a digital balance (VL-3200H, Shimadzu, USA). Heart rate and mean arterial pressure (MAP) measurements were performed by the tail plethysmography method (LE5001®, Panlab, Spain).

2.5. Food and caloric intake

Metabolic assays were performed for evaluation of food and caloric intake, which consisted in placing the experimental animals in individual metabolic cages for two days, with free access to water and feed. The rats were placed in the metabolic cages one day after the training session; the first day was considered as acclimation period and on the second day the data used for the analysis were collected. This was defined from a pilot study, where we observed that the food consumption of the animals did not vary significantly between the second day and the five subsequent days of metabolic assay. Food intake (g/day) was determined by the difference between the initial feed amount and leftovers at the end of 24 h; metabolizable energy intake (Kcal/day) was calculated based on the diet's energy density.

2.6. Glucose tolerance test

The animals, after 12-h food deprivation, underwent a small section at the distal end of their tail for blood collection and evaluation of glycemia, initially at time zero (fasting glycemia) and, subsequently, at 30, 60 and 90 min after intraperitoneal injection of 50% glucose solution at a dose of 1 g/Kg body weight. Blood glucose levels were determined using a manual digital glucometer with appropriate reagent strips (Accu-check® Performa, Roche®, Germany). Following the glucose tolerance test, the glucose tolerance curve of each animal was constructed and then the area under the curve (AUC) was calculated on GraphPad Prism® 5 (GraphPad Software, Inc., USA).

2.7. Animals euthanasia and obtaining serum and tissue samples

Twenty-four hours after the last training session, the animals were euthanized by decapitation. Trunk blood was collected in 1.5 mL dry microtubes and centrifuged (14,000 rpm at 4 °C for 15 min) for serum separation, and was stored in a −70 °C freezer until biochemical, hormonal and inflammatory parameter measurement. Soleus and gastrocnemius muscles of both hind limbs were dissected, weighed, identified, frozen in liquid nitrogen and stored in a −70 °C freezer for molecular biology assays. The animals were submitted to median laparotomy for collection and quantification of subcutaneous (SAT), epididymal (EAT), retroperitoneal (RAT) and mesenteric (MAT) adipose tissue deposits, as well as brown adipose tissue (BAT). The adiposity index was calculated from the visceral adipose tissue (VAT = EAT + RAT + MAT) [16]:

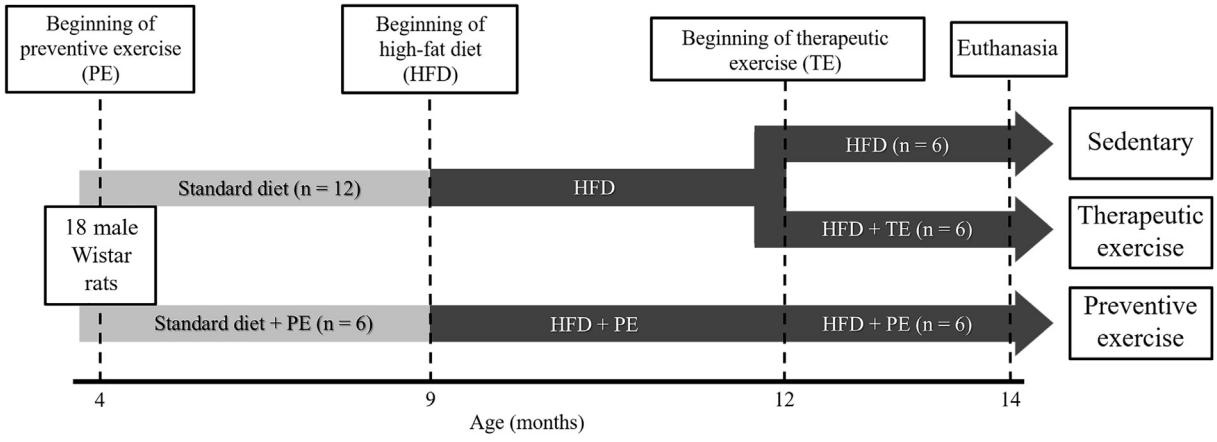

Fig. 1. Experimental flow chart of the study.

$$\text{Adiposity index } (\%) = \frac{\text{VAT}}{\text{Body weight} - \text{VAT}} \times 100$$

2.8. Plasmatic biochemical parameters

Serum concentrations of total cholesterol, HDL cholesterol, triglycerides, and NEFA were measured using commercial colorimetric kits according to manufacturers' instructions (Monoreagent Cholesterol – K083, Enzymatic HDL Cholesterol – K015 and Triglicerides Monoreagent – K117, Bioclin/Quibasa Química Básica, Brazil; NEFA FA 115, Randox, UK). Absorbance readings for total cholesterol, HDL cholesterol and triglycerides were made by spectrophotometry (SP 2000 UV, BEL® Photonics, Brazil); NEFA was dosed using an automatic biochemical analyzer (Miura, Kovalent, Germany). LDL cholesterol was estimated according to the following equation [17]:

$$\text{LDL} = \text{Total cholesterol} - \text{HDL} - \frac{\text{triglycerides}}{5}$$

2.9. Plasmatic hormonal and inflammatory parameters

Plasmatic levels of insulin and TNF-α were quantified by the enzyme-linked immunosorbent assay (ELISA) technique using commercial solid-phase sandwich kits, according to the manufacturers' instructions (Insulin: Millipore, Germany; TNF-α: Novex®, Life Technologies, USA). ELISA reaction readings were performed on a microplate spectrophotometer (VersaMax, Molecular Devices, USA).

2.10. Insulin resistance, insulin sensitivity and functional capacity of pancreatic β cells

To estimate insulin resistance and insulin sensitivity, respectively, the Homeostatic Model Assessment – Insulin Resistance (HOMA-IR) [18] and Quantitative Insulin Sensitivity Check Index (QUICKI) [19] were calculated, and the Homeostatic Model Assessment – β cell (HOMA-β) was used to evaluate the function of pancreatic β cells [18]:

$$\text{HOMA} - \text{IR} = \frac{\text{Fasting insulin}\left(\frac{\mu\text{UI}}{\text{mL}}\right) \times \text{fasting glucose}\left(\frac{\text{mmol}}{\text{L}}\right)}{22},5$$

$$\text{QUICKI} = \frac{1}{\log[\text{fasting insulin}(\mu\text{UI/mL})] + \log[\text{fasting glucose}(\text{mg/dL})]}$$

$$\text{HOMA} - \beta = \frac{20 \times \text{fasting insulin}\left(\frac{\mu\text{UI}}{\text{mL}}\right)}{\text{fasting glucose}\left(\frac{\text{mmol}}{\text{L}}\right) - 3,5}$$

2.11. Gene expression of insulin-dependent, insulin-independent and insulin resistance pathways

2.11.1. RNA extraction

Gastrocnemius and soleus muscle samples were homogenized in QIAzol® lysis reagent using the TissueLyser II system (Qiagen, USA). Total RNA was extracted using the RNeasy Microarray Tissue Mini kit (Qiagen, USA), according to the manufacturer's instructions. During the purification process, RNA was treated with RNase-Free DNase (Qiagen, USA) for digestion of DNA fragments. Concentration (ng/μL) and purity of total RNA were assessed by reading the absorbance (A_{260}:A_{230} and A_{260}:A_{280}) in spectrophotometer (NanoDropTM 2000, Thermo Fisher Scientific, USA). The RNA was maintained at −70 °C until the time of reverse transcription.

2.11.2. Complementary DNA (cDNA) synthesis and real time quantitative polymerase chain reaction (qPCR)

The cDNA was synthesized from 1 μg of total RNA by using the RT^2 First Strand kit (Qiagen, USA) according to the manufacturer's instructions. Samples were stored at −70 °C until the real time qPCR. Prior to performing qPCR, the integrity and quality of RNA and cDNA were tested using the RT^2 RNA QC PCR Array system (Qiagen, USA), which allows the detection and prevention of reverse transcription inhibition, polymerase chain reaction (PCR) amplification inhibition, genomic DNA contamination, false positive signals, and multiple peak dissociation curves. For real time qPCR analysis the RT^2 SYBR Green Mastermixes were used in combination with RT^2 Profiler Custom PCR Arrays (Qiagen, USA) to simultaneously examine the expression levels of twelve target genes [INSR (insulin receptor), IRS1 (insulin receptor substrate 1), IRS2 (insulin receptor substrate 1), PIK3R1 (phosphoinositide-3-kinase, regulatory subunit 1), PIK3CA (phosphoinositide-3-kinase, catalytic subunit), AKT1 (v-akt murine thymoma viral oncogene homolog 1 or protein kinase B - isoform 1), AKT2 (v-akt murine thymoma viral oncogene homolog 2 or protein kinase B – isoform 2), PRKAA1 (protein kinase, AMP-activated – AMPK, alpha 1 catalytic subunit), TBC1D1 (TBC1 domain family, member 1), SLC2A4 (solute carrier family 2, member 4 or glucose transporter type 4 – GLUT4), IKBKB (inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta or beta kinase from IkB) and MAPK8 (mitogen-activated protein kinase 8 or N-terminal c-Jun kinase – JNK)] and two reference genes [ACTB (actin, beta) and GAPDH (glyceraldehyde-3-phosphate dehydrogenase)] in 96-well plates; the plates also contained a genomic DNA control (GDC), a reverse transcription control (RTC) and a positive PCR control (PPC). All procedures were conducted according to the manufacturer's instructions and thermocycling was performed with the StepOne Plus thermal cycler (Applied Biosystem, USA). Gene expression was normalized by the GAPDH, for the gastrocnemius muscle, and ACTB genes, for the soleus muscle. From results of Ct (cycle threshold), the fold change was calculated using comparative method ($2^{-\Delta\Delta Ct}$).

2.12. Statistical analysis

Results were expressed as means ± standard deviations and medians ± interquartile ranges. Normality was tested by Shapiro–Wilk test, and homoscedasticity by Levene test. To evaluate the group factor effect, one-way analysis of variance (ANOVA) was used, with multiple comparisons performed by means of post hoc Tukey's test. When normality and/or homoscedasticity were violated, we proceeded with non-parametric statistics: Kruskal–Wallis test, with comparisons between pairs verified by Mann–Whitney test. In all analyses, level of significance was 5% ($\alpha = 0.05$). Data were analyzed in IBM SPSS Statistics for Windows (IBM SPSS, 21.0, 2012, Armonk, NY: IBM Corp.).

2.13. Ethical statements

This study was carried out in accordance with norms of the National Council of Control of Animal Experimentation (Ministry of Science and Technology, Brazil). All experimental procedures were

approved by the IMS/UFBA Animal Use Ethics Committee (protocol: 011/2014).

3. Results

3.1. Cardiovascular parameters, food and caloric intake, body weight and body composition parameters

As shown in Fig. 2, at the end of experiment both the trained groups had higher food and caloric intake, in relation to the sedentary group; however, the effect on these variables was higher for the group that underwent preventive exercise. Only the preventive exercise group presented lower heart rate, MAP, adiposity index, SAT, RAT and EAT, in addition to greater muscle mass of hind limbs. There were no therapeutic or preventive effects of exercise on body weight and content of MAT and BAT.

3.2. Plasmatic biochemical and inflammatory parameters

Compared to the sedentary group, both trained groups had, similarly, lower total cholesterol/HDL and LDL/HDL ratios, as well as reduced plasma TNF-α concentration. Nevertheless, only the group that engaged in preventive exercise presented lower levels of total cholesterol and triglycerides, and lower TG/HDL ratio. There were no therapeutic and preventive effects of exercise on HDL cholesterol, LDL cholesterol and NEFA (Table 2).

3.3. Glucose tolerance, plasma insulin, insulin resistance, insulin sensitivity and functional capacity of β cells

Results of analyses of data related to glucose homeostasis are presented in Fig. 3. The results of the glucose tolerance test indicated that only the preventive exercise group had lower glycemic levels than the sedentary group at zero (fasting), 15, 30 and 60 min; however, at 90 min after the glucose injection, the two groups subjected to exercise (therapeutic and preventive) presented similarly lower blood glucose levels (Fig. 3A). Analysis of other parameters (Fig. 3B–F) showed that only the preventive exercise group had lower AUC for the glucose tolerance test, plasma insulin and HOMA-IR, and higher QUICKI, in relation to the sedentary group. Therapeutic and preventive effects of exercise on pancreatic β cell function were not observed.

3.4. Gene expression of insulin-dependent, insulin-independent and insulin resistance pathways in skeletal muscle

The analyses of gene expression in gastrocnemius and soleus muscles are presented in Fig. 4. The results for gastrocnemius showed that only the group that underwent preventive exercise had up-regulation of INRS, IRS1, IRS2, PIK3CA, AKT1, TBC1D1 and SLC2A4 in relation to the sedentary group; however, both exercise regimens (therapeutic and preventive) were responsible for IKBKB down-regulation. No effect of exercise was observed on PIK3R1, AKT2, PRKAA1 and MAPK8 expression in the gastrocnemius (Fig. 4A). The data from the soleus indicated that only the preventive exercise group presented up-regulation of INRS, IRS1, IRS2, PI3KCA, AKT1, AKT2, PRKAA1 and SLC2A4, and MAPK8 down-regulation, compared to the sedentary group. In addition, both exercises (therapeutic and preventive) were responsible for up-regulation of TBC1D1 and down-regulation of IKBKB, and the effect on the gene expression of TBC1D1 was greater in the preventive exercise group. No effect of exercise on PIK3R1 gene expression was observed in the soleus (Fig. 4B).

4. Discussion

To the best of our knowledge, this was the first study investigating and comparing the short and long-term effects of aerobic exercise on cardiometabolic disease risk parameters in a simultaneous aging and diet-induced obesity experimental model. Our findings confirmed the hypothesis that regular practice of lifelong aerobic exercise (i.e., preventive exercise) was more advantageous than a short-term training protocol (i.e., therapeutic exercise) to reduce the cardiometabolic risk factors associated with aging and diet-induced obesity.

Preventive exercise promoted benefits in cardiovascular function and body composition, greater improvement in plasma lipid profile and glucose tolerance, and improved insulin resistance and insulin sensitivity due to up-regulation of insulin-dependent and insulin-independent pathway gene expression, and down-regulation of genes that inhibit insulin action.

In contrast to previous reports involving diet-induced obese young rats [13,20], we found no evidence of improvement mediated by short-term treatment of moderate intensity aerobic exercise on cardiovascular parameters, body composition, insulin resistance and insulin sensitivity. It is possible that volume/intensity relation for the execution time of our therapeutic exercise protocol did not provide sufficient stimulus to deal with cardiometabolic dysfunctions due to aging and continued consumption of high-fat diet. Perhaps a short-term exercise protocol associated with dietary intervention or high intensity training (e.g., high intensity interval training) could be more efficient; these hypotheses should be addressed in future studies. Therapeutic and preventive exercises were comparable in their ability to improve total cholesterol/HDL and LDL/HDL ratios, systemic inflammation and final glucose tolerance (i.e., after 90 min of glucose load infusion). Furthermore, both treatments significantly increased food and caloric intake, with greatest effect in preventive exercise group.

Aging and obesity are strongly associated with the development of cardiovascular problems [9,21]. In our study, we observed that, compared to sedentary controls, the preventive exercise group had a reduction in heart rate and protection against increased MAP. A study with aged rats showed that increased blood pressure might be related to increased body adiposity and insulin resistance [22]; therefore, it is likely that improvement in body composition and insulin sensitivity observed in the preventive exercise group was responsible for inhibiting mechanisms of sustained increase in blood pressure. In addition, other studies have demonstrated in diet-induced obese and spontaneously hypertensive eutrophic rats that exercise may act through various mechanisms to reduce heart rate and improve blood pressure control, which include benefits in vagal modulation, changes in cardiac geometry, increased vasodilation by endothelial vasoactive factors release, capillary angiogenesis, and reduction of oxidative stress, among others [23–25].

In the present study, we verified that, despite being accompanied by an increase in caloric intake, therapeutic and preventive exercises did not lead to a change in body weight. Nevertheless, preventive exercise resulted in better body composition at the end of the experiment since it significantly attenuated the accumulation of subcutaneous and intra-abdominal adipose tissue, and loss of muscle mass. Unlike what has been observed in diet-induced obese young rats [13], our model indicated that exercise led to a compensatory increase in caloric intake, and that this response was more pronounced in the preventive exercise group. It has already been demonstrated in rodents that exercise is directly responsible for increasing total

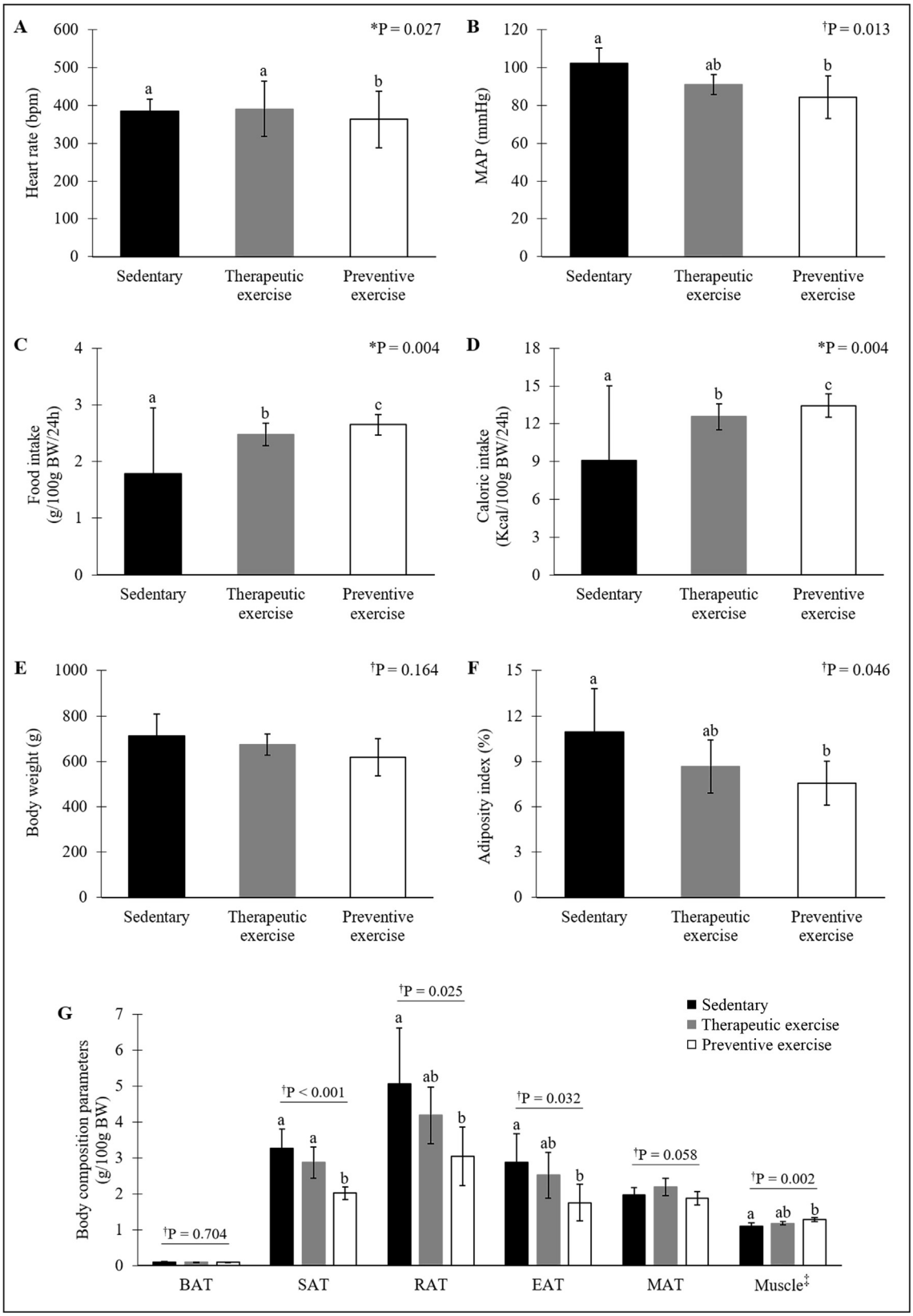

Fig. 2. Cardiovascular parameters, food and caloric intake, body weight and body composition parameters of aging and obese experimental groups. A, heart rate; B, mean arterial pressure (MAP); C, food intake; D, caloric intake; E, body weigh; F, adiposity index; G, body composition parameters. BW, body weight; BAT, brown adipose tissue; SAT, subcutaneous adipose tissue; RAT, retroperitoneal adipose tissue; EAT, epididymal adipose tissue; MAT, mesenteric adipose tissue. * Kruskal–Wallis test (comparisons between pairs by Mann–Whitney test); † one-way ANOVA (multiple comparisons by Tukey's test); [a,b,c] different letters indicate statistical difference ($p \leq 0.05$) between the groups. The results were expressed as median ± interquartile range (A, C e D) and mean ± standard deviation (B, E, F e G). ‡ Sum of right and left soleus and gastrocnemius muscles.

Table 2
Plasmatic biochemical parameters of aging and obese experimental groups.

Variable	Sedentary	Therapeutic exercise	Preventive exercise	P-value*
Total cholesterol (mg/dL)	96.38 ± 11.72[a]	93.05 ± 18.46[ab]	74.95 ± 5.78[b]	0.033
HDL cholesterol (mg/dL)	24.79 ± 7.87	30.52 ± 9.95	42.73 ± 15.22	0.060
LDL cholesterol (mg/dL)	46.83 ± 10.97	34.47 ± 18.11	24.67 ± 15.14	0.090
NEFA (mmol/L)	0.48 ± 0.13	0.40 ± 0.09	0.35 ± 0.05	0.106
Triglycerides (mg/dL)	92.04 ± 17.61[a]	100.56 ± 22.17[a]	59.64 ± 14.73[b]	0.005
Total cholesterol/HDL	3.79 ± 1.01[a]	2.50 ± 0.53[b]	2.22 ± 0.59[b]	0.007
LDL/HDL	2.02 ± 0.88[a]	0.97 ± 0.55[b]	0.74 ± 0.57[b]	0.016
Triglycerides/HDL	3.80 ± 0.69[a]	3.02 ± 0.41[a]	1.80 ± 0.57[b]	<0.001
TNF-α (pg/mL)	8.79 ± 2.15[a]	3.02 ± 2.36[b]	2.27 ± 0.58[b]	<0,001

NEFA, non-esterified fatty acids.
*One-way ANOVA (multiple comparisons by Tukey's test); [a,b] different letters indicate statistical difference ($p \leq 0.05$) between the groups. The results were expressed as mean ± standard deviation.

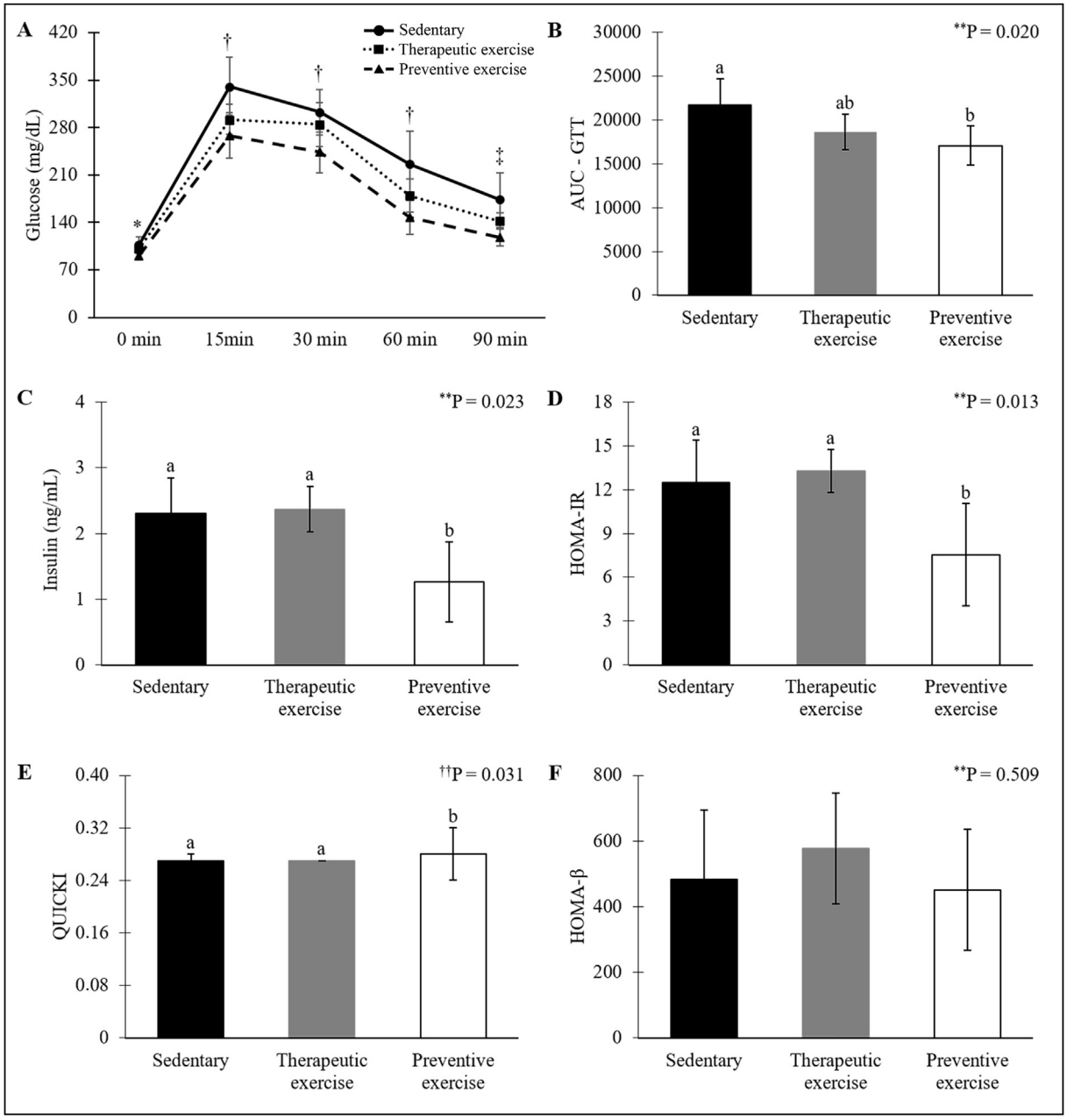

Fig. 3. Glycemic homeostasis' parameters of aging and obese experimental groups. A, glucose tolerance test (GTT); B, area under the curve (AUC) of GTT; C, plasmatic insulin; D, Homeostatic Model Assessment – Insulin Resistance (HOMA-IR); E, Quantitative Insulin Sensitivity Check Index (QUICKI); F, Homeostatic Model Assessment – β cell (HOMA-β). * Kruskal–Wallis test (comparisons between pairs by Mann–Whitney test: preventive exercise ≠ sedentary and therapeutic exercise); † one-way ANOVA (multiple comparisons by Tukey's test: preventive exercise ≠ sedentary); ‡ Kruskal–Wallis test (therapeutic exercise and preventive exercise ≠ sedentary); ** one-way ANOVA; †† Kruskal–Wallis test; [a,b] different letters indicate statistical difference ($p \leq 0.05$) between the groups. The results were expressed as mean ± standard deviation (A, B, C, D and F) and median ± interquartile range (E).

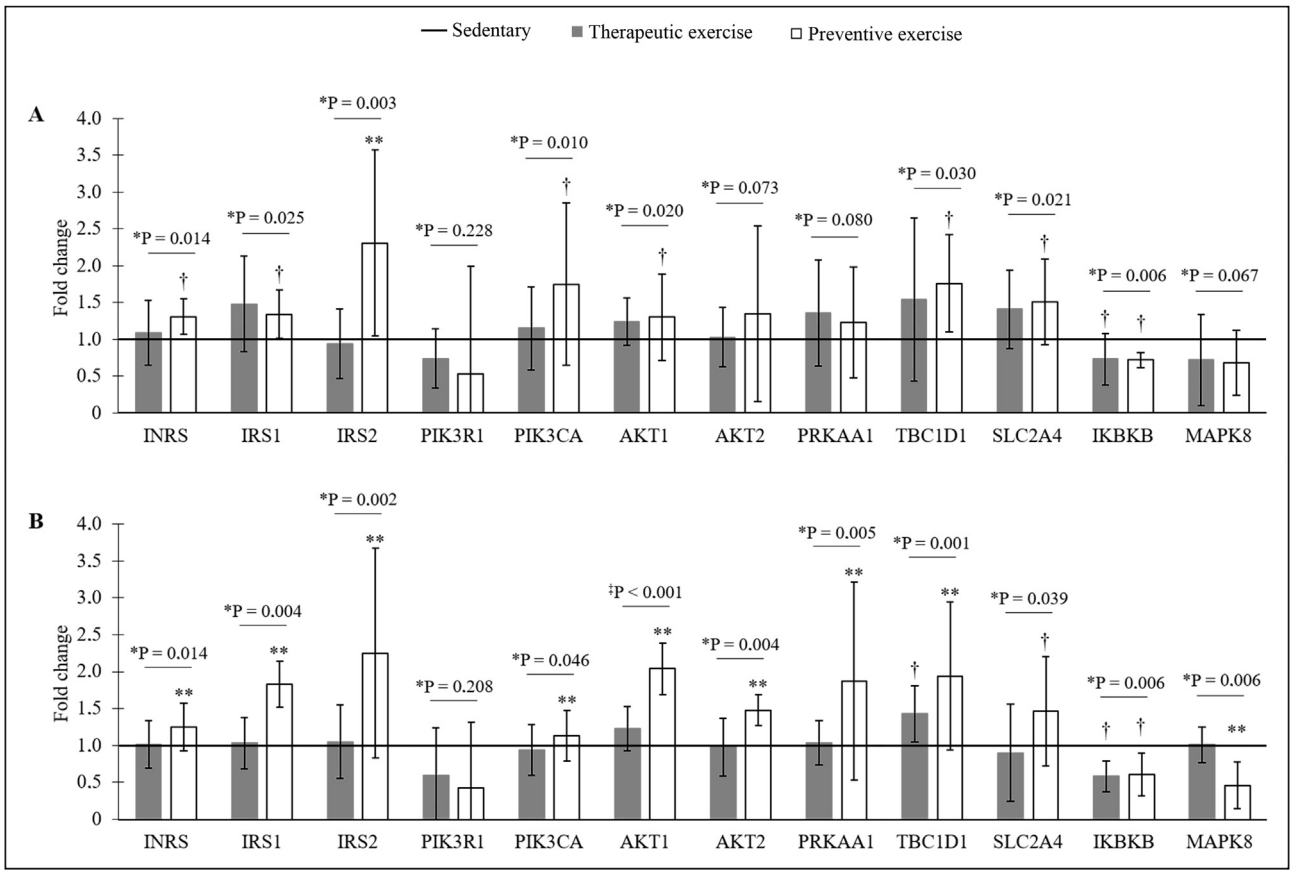

Fig. 4. Gene expression in the gastrocnemius (A) and soleus (B) muscles of the aging and obese experimental groups. INSR, insulin receptor; IRS1, insulin receptor substrate 1; AKT1, v-akt murine thymoma viral oncogene homolog 1 or protein kinase B; PRKAA1, protein kinase, AMP-activated − AMPK, alpha 1 catalytic subunit; SLC2A4, solute carrier family 2, member 4 or glucose transporter type 4 (GLUT4); IKBKB, inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta or beta kinase from IkB; MAPK8, mitogen-activated protein kinase 8 or N-terminal c-Jun kinase (JNK). * Kruskal–Wallis test (comparisons between pairs by Mann–Whitney test); ‡ one-way ANOVA (comparisons between pairs by Tukey's test); † statistically different from sedentary; ** statistically different from sedentary and therapeutic exercise. The results were expressed as median ± interquartile range, except for expression of AKT1 in the soleus which was expressed as mean ± standard deviation.

energy expenditure and spontaneous physical activity in active and inactive cycles [13]. This possibly explains how exercise, even with the increased high-fat diet consumption, was able to prevent a positive energetic balance, improving body composition in our preventive exercise group.

Changes in body composition inherent to aging and ingestion of a high-fat diet contribute directly to the development of dyslipidemic status and systemic inflammation [4,7,26]. Our results for plasma lipid measurements, lipoprotein ratios and inflammatory marker indicated that preventive exercise provided protection against increased total cholesterol, triglycerides, total cholesterol/HDL, LDL/HDL and triglycerides/HDL ratios, and plasma TNF-α; in turn, therapeutic training contributed to a reduction in cardiovascular risk by reducing total cholesterol/HDL and LDL/HDL ratios, and plasma TNF-α. Preventive effects of exercise on plasma lipemia and inflammation possibly occurred in response to improved body composition. However, as observed in the therapeutic exercise group, exercise also seems to act independently of body adiposity reduction. Mechanisms of exercise's independent effect on plasma lipemia are not very clear and need to be better investigated; however, there is evidence in humans that muscle contraction can modulate blood biochemistry by increasing the activity of the lipoprotein lipase enzyme, and the use of triglycerides as an energy source and for muscle fiber repair; moreover, it could reduce the activity of the esterified cholesterol transfer protein [27]. Otherwise, the anti-inflammatory mechanisms, independent of body fat reduction, triggered by exercise are relatively well known and involve an increase of muscle IL-6 (which has an anti-inflammatory action in this tissue), reduction of oxidative stress, activation of the parasympathetic nervous system (cholinergic anti-inflammatory reflex), activation of the hypothalamic-pituitary-adrenal axis (increased secretion of cortisol, known to have a potent anti-inflammatory effect) and toll-like receptor 4 (TLR-4) negative regulation, which is involved in activation of several inflammatory cascades [28].

It is well established that both aging and obesity are strong triggers of glycemic metabolism disorders and that exercise is known to improve insulin resistance and insulin sensitivity in humans and animals [1,12,13]. We found that both therapeutic and preventive exercises improved glucose tolerance, but only preventive exercise improved insulin resistance and insulin sensitivity, and reduced plasma insulin levels. The effects observed in the preventive exercise group can be attributed, in part, to increased muscle mass, besides lower body adiposity and consequent reduction of systemic inflammation. However, the fact that this treatment did not affect circulating NEFA and that the therapeutic training promoted some improvement in glucose tolerance independently of fat mass reduction suggests that a large part of the effects mediated by exercise were due to specific improvements in skeletal muscle.

Our gene expression analysis in the studied muscles agrees with the hypothesis described above, since the preventive exercise up-regulated insulin signaling pathway genes (INRS, IRS1, IRS2, PIK3CA, AKT1, AKT2, TBC1D1, and SLC2A4) and down-

regulated insulin resistance genes (IKBKB and MAPK8). The effect of preventive exercise on PRKAA1 up-regulation only in the soleus indicates that long-term training can also modulate glucose uptake into skeletal muscle by the insulin-independent pathway (AMPK pathway); in addition, it suggests that the AMPK pathway is more responsive to exercise in muscles with predominantly type I slow-twitch fibers with oxidative characteristics (e.g., soleus) than in muscles with a predominance of type IIB fast-twitch fibers with glycolytic characteristics (e.g., gastrocnemius). Another point to be addressed is that IKBKB down-regulation and low up-regulation of insulin-dependent pathway in the therapeutic exercise group suggest that short-term effects of exercise happened by means of IKKB/NF-kB inflammatory pathway inhibition. In turn, the long-term effects possibly involved several mechanisms such as inflammatory pathways inhibition (IKKB/NF-kB and JNK pathways), improvement in insulin signaling and insulin-independent pathway activation.

A limitation of this study was that differences in gene expression observed in comparison to the sedentary controls were not validated with protein measurements. In addition, it should be noted that this study was conducted in aging male rats, all fed with high-fat diet; therefore, the way the results translate into other animal models or humans is unknown.

In conclusion, our results provided evidence that early and continued practice of moderate aerobic exercise benefits cardiometabolic health during the simultaneous aging process and development of obesity. On the other hand, short-term protocols may not offer sufficient therapeutic responses against cardiometabolic disorders induced by aging and obesity in a condition of continuous ingestion of a high-fat diet.

Statement of authorship

RSC made contributions to conception and design of the study, data acquisition, analysis and interpretation, and drafting the article. TJS made contributions to interpretation of data and revised the manuscript critically for important intellectual content. RP made contributions to conception and design of the study, and revised the manuscript critically for important intellectual content. TMLC made contributions to data acquisition, data analysis and revised the manuscript critically for important intellectual content. DSOC, MVO and LMM made contributions to data acquisition and revised the manuscript critically for important intellectual content. CKCS revised the manuscript critically for important intellectual content. ACMMG made contributions to conception and design of the study, data acquisition and interpretation, and revised the manuscript critically for important intellectual content. All authors approved the final version of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnesp.2018.10.003.

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