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Exercise-induced changes on exerkines that might influence brown adipose tissue metabolism in young sedentary adults

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ABSTRACT

In rodents, exercise alters the plasma concentration of exerkines that regulate white adipose tissue (WAT) browning or brown adipose tissue (BAT) metabolism. This study aims to analyse the acute and chronic effect of exercise on the circulating concentrations of 16 of these exerkines in humans. Ten young sedentary adults (6 female) performed a maximum walking effort test and a resistance exercise session. The plasma concentration of 16 exerkines was assessed before, and 3, 30, 60, and 120 min after exercise. Those exerkines modified by exercise were additionally measured in another 28 subjects (22 women). We also measured the plasma concentrations of the exerkines before and after a 24-week exercise programme (endurance + resistance; 3-groups: control, moderate-intensity and vigorous-intensity) in 110 subjects (75 women). Endurance exercise acutely increased the plasma concentration of lactate, norepinephrine, brain-derived neurotrophic factor, interleukin 6, and follistatin-like protein 1 (3 min after exercise), and musclin and fibroblast growth factor 21 (30 and 60 min after exercise), decreasing the plasma concentration of leptin (30 min after exercise). Adiponectin, atrial natriuretic peptide (ANP), β-aminoisobutyric acid, meteorin-like, follistatin, pro-ANP, irisin and myostatin were not modified or not detectable. The resistance exercise session increased the plasma concentration of lactate 3 min after exercise. Chronic exercise did not alter the plasma concentration of these exerkines. In sedentary young adults, acute endurance exercise releases to the bloodstream exerkines that regulate BAT metabolism and WAT browning. In contrast, neither a low-volume resistance exercise session nor a 24-week training programme modified plasma levels of these molecules.

KEYWORDS
Beige fat; brown fat; exerkines; thermogenesis

Highlights

- Acute endurance exercise increases the plasma concentration of lactate, norepinephrine, brain-derived neurotrophic factor, interleukin 6, follistatin-like protein 1, musclin, and fibroblast growth factor 21, and decrease the plasma concentration of leptin.
- The exercise-induced change in lactate plasma concentration is positively associated with brown adipose tissue volume, glucose uptake and radiodensity.
- Neither acute resistance exercise nor chronic exercise significantly alter the plasma concentration of these exerkines.

Trial registration: ClinicalTrials.gov identifier: NCT02365129.

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1. Introduction

Brown adipose tissue (BAT) can produce heat to maintain homeostatic body temperature, mainly through the action of the uncoupling protein 1 (UCP1) (Cannon & Nedergaard, 2004). Another type of adipocytes, beige adipocytes, that present brown-like characteristics such as enrichment of mitochondria and UCP1 expression can be recruited in a process known as white adipose tissue (WAT) browning (Petrovic et al., 2010). Since brown and beige adipocytes have been found to be present and metabolically active in adult humans (Cypess et al., 2009), BAT activation and WAT browning are considered promising therapeutic avenues for metabolic diseases treatment (Villarroya et al., 2019).

In murine models, exercise seems to induce WAT browning (Vidal & Stanford, 2020), although the underlying mechanisms are still partially unknown. Many of the endocrine signalling molecules secreted by several tissues in response to exercise, so-called exerkines, have been shown to induce WAT browning and/or regulate BAT metabolism (Mendez-Gutierrez, Osuna-Prieto, Aguilera, Ruiz, & Sanchez-Delgado, 2020). These molecules include adiponectin, leptin, atrial natriuretic peptide (ANP), pro-ANP, β-aminoisobutyric acid (BAIBA), lactate, norepinephrine, brain-derived neurotrophic factor (BDNF), interleukin 6 (IL6), meteorin-like, follistatin, follistatin-like 1 (FSTL1), irisin, myostatin, musclin, and fibroblast growth factor 21 (FGF21) (Mendez-Gutierrez et al., 2020). Following preclinical evidence, it could be hypothesised that exercise also induces WAT browning and/or regulate BAT metabolism in humans. Unfortunately, the available evidence in humans is insufficient and controversial, mainly due to the lack of longitudinal studies with adequate statistical power. Moreover, the technological limitations for assessing BAT volume and activity in humans also pose a severe restriction to the advance in the field (Acosta et al., 2018; Dinas et al., 2017). One of the issues that remains to be addressed is characterising the acute and chronic effect of different types of exercise (i.e. endurance and resistance exercise) on the circulating levels of exerkines that are known to induce WAT browning and/or regulate BAT metabolism in humans.

This study aims to analyse the acute (endurance and resistance) and chronic (combined endurance and resistance) effect of exercise on the circulating concentrations of 16 exerkines that have been shown to regulate BAT metabolism and/or WAT browning *in vitro* or in preclinical models (adiponectin, leptin, ANP, pro-ANP, BAIBA, lactate, norepinephrine, BDNF, IL6, meteorin-like, follistatin, FSTL1, irisin, myostatin, musclin, and FGF21), in a cohort of young sedentary adults. We also explored the associations between the exercise-induced changes in the concentration of these exerkines and BAT volume, glucose uptake and mean radiodensity, assessed after an individualised cold exposure. Moreover, we explored whether the exercise-induced changes in the circulating exerkines are related to body composition, sex, circulating cardiometabolic risk factors, or physical fitness.

2. Material and methods

2.1. Study design

This work has been conducted under the framework of the ACTIBATE study (Sanchez-Delgado et al., 2015), a randomised controlled trial designed to study the effect of an exercise programme on BAT volume and activity (ClinicalTrials.gov ID: NCT02365129). Inclusion criteria were 18–25 years old, reporting no more than 20 min of moderate-vigorous physical activity in a maximum of 3 days per week, absence of cardiometabolic disease, non-smoker, not taking any medication affecting energy metabolism, and having stable body weight during the previous three months. This study was conducted following the last version of Declaration of Helsinki. The protocol and written informed consent were approved by the Ethics Committee on Human Research of the University of Granada (n° 924) and "Servicio Andaluz de Salud".

First, we analysed the acute effect of endurance and resistance exercise on the circulating levels of 16 exerkines in 10 participants (4 men, 6 women) before and 3, 30, 60 and 120 min after a single exercise bout, onwards referred as *discovery study*. Those exerkines that were changed by acute endurance exercise were further studied in another 28 participants only before exercise and at the time point when the molecule was altered in the *discovery study*. These individuals, together with the 10 participants of the *discovery study* resulted in a total of 38 participants (10 men, 28 women), onwards referred as *confirmatory study*. In this group, we also analysed the association between the exercise-induced change in the selected molecules and BAT-related variables, body composition, cardiometabolic risk factors, and physical fitness. Finally, we tested the effect of a 24-week exercise training programme, including endurance and resistance exercise, on the circulating levels of 16 exerkines using samples collected before and after the exercise programme in 110 participants (35 men, 75 women) during resting and fasting conditions, onwards referred as *chronic study*. The study design is summarised in Supplementary Figure 1. Descriptive

characteristics of the participants are presented in Table 1. The data presented in this table have been obtained in the pre-intervention of the chronic study. The training programme was performed in four different waves (both in 2015 and 2016): (i) from September to April, (ii) from October to April, (iii) from October to May, and (iv) from November to May.

2.2. Acute exercise sessions

Plasma samples for the *discovery and confirmatory studies* were collected during 2 exercise sessions, one involving endurance exercise and the other resistance exercise, taking place during the baseline assessment period of the ACTIBATE study. Both sessions were carried out in 3–5 h fasted state after avoiding stimulants as well as moderate (24 h before) and vigorous (48 h before) exercise (Sanchez-Delgado et al., 2015).

The endurance exercise session consisted of a maximum effort test on a treadmill (Pulsar treadmill, H/P/Cosmos Sports & Medical GmbH, Nussdorf-Traunstein, Germany) as previously described (Martinez-Tellez, Sanchez-Delgado, Amaro-Gahete, Acosta, & Ruiz, 2019). For warming up, participants walked at 3 km/h for 1 min and at 4 km/h for 2 min (0% grade). Subsequently, the test started by walking at 5.3 km/h and 0% grade. From that moment on, the treadmill grade was increased by 1% every minute, until volitional exhaustion was reached. Immediately after, participants started a 5-minute recovery. Participants were equipped with a heart rate monitor (Polar RS800CX, Polar Electro Öy, Kempele, Finland), 10 electrodes for electrocardiogram monitoring, and a Hans-Rudolph plastic mask (model 7400, Hans Rudolph Inc., Kansas City, MO, USA) connected to a preVent™ metabolic flow sensor (Medical graphics Corp, St Paul, MN, USA) for respiratory gas exchange analyses using a CPX Ultima CardioO2 gas exchange analysis system (Medical Graphics Corp, St Paul, MN, USA).

The resistance exercise session consisted of a maximum isometric strength test in leg press, a handgrip strength test, and two one repetition maximum (1-RM) tests in bench and leg press, as described elsewhere (Martinez-Tellez et al., 2019). Participants first

Table 1. Characteristics of the study participants.

	Acute effect Discovery study ($n = 10$)		Acute effect Confirmatory study ($n = 38$)		Chronic effect Chronic study ($n = 110$)	
	Mean	SD	Mean	SD	Mean	SD
Demographics						
Sex (Male/Female)	4/6		10/28		35/75	
Age (years)	21.5	2.3	22.1	2.4	22.1	2.2
Body composition						
Weight (kg)	68.0	14.1	66.2	12.4	70.2	15.3
Height (cm)	168.9	8.9	166.4	8.0	167.9	8.5
Body mass index (kg/m^2)	23.7	3.5	23.9	3.9	24.7	4.2
Waist circumference (cm)	76.1	9.1	77.5	11.2	81.4	12.9
Lean mass (kg)	41.1	8.1	39.6	7.8	41.5	9.3
Fat mass (kg)	23.3	6.8	23.1	8.7	24.9	8.5
Fat mass (%)	34.7	5.2	35.0	9.0	34.8	7.5
VAT mass (g)	294	96	311	176	346	176
Cardiometabolic risk factors						
Glucose (mg/dL)	85.6	6.9	87.2	5.8	87.2	6.3
Insulin (µUl/mL)	6.1	2.7	7.4	4.1	8.2	4.1
HOMA-IR	1.3	0.6	1.6	1.0	1.8	1.0
Triacylglycerol (mg/dL)	68.2	17.4	89.0	70.0	83.1	47.3
Cholesterol (mg/dL)	168.7	24.3	171.1	41.8	162.7	30.6
HDL cholesterol (mg/dL)	53.6	8.1	53.3	9.5	52.5	11.9
LDL cholesterol (mg/dL)	101.5	22.1	102.1	32.9	94.3	25.7
Systolic BP (mmHg)	114.7	11.2	114.8	10.5	117.5	11.6
Diastolic BP (mmHg)	71.2	9.9	70.7	7.7	71.4	7.3
Physical fitness						
Time to exhaustion (min)	16.3	3.5	14.6	3.94	15.3	3.4
VO_2peak (mL/kg/min)	42.4	7.2	41.9	8.0	40.9	8.3
Hand grip strength (kg)	35.1	9.9	31.2	7.6	31.1	7.5
RM leg press (kg)	222.8	50.5	204.3	50.2	197.8	64.1
RM bench press (kg)	35.9	11.5	30.4	10.5	31.1	14.4
Brown adipose tissue						
BAT volume (mL)	70.5	62.8	71.8	45.8	69.1	55.8
BAT SUVmean	3.5	2.4	4.0	1.9	3.8	1.9
BAT SUVpeak	11.3	8.7	12.6	9.0	11.1	8.2
BAT mean radiodensity (HU)	−59.4	12.0	−58.5	11.8	−59.9	11.4

Data presented as mean and standard deviation (SD), except for sex. Abbreviations: VAT: visceral adipose tissue; HOMA-IR: homeostasis model assessment of insulin resistance; HDL: high-density lipoproteins; LDL: low-density lipoproteins; BP: blood pressure; VO_2peak: Maximal oxygen uptake; RM: 1 Repetition Maximum; BAT: Brown adipose tissue; SUV: Standardised uptake value; HU: Hounsfield units.

completed the maximum isometric strength test in leg press, performing two 3-second repetitions, two minutes apart. Later, participants performed the handgrip strength test by completing two repetitions with each hand, one minute apart. Then, participants completed the RM test in the leg press machine and the bench press (Power rack, Model 3111, Keiser Corporation, Fresno CA, USA). After warming up, they were instructed to perform one set of 8 repetitions selecting the resistance with which they could perform 15 repetitions as much. After a 1-minute recovery, the resistance load was increased by the study personnel, aiming to set a load with which the participant could perform <10 repetitions and they did as many repetitions as possible. The maximum number of attempts was three. A more detailed description can be found elsewhere (Martinez-Tellez et al., 2019).

Before each exercise session, an intravenous catheter was inserted in the antecubital vein. Blood was collected in 2 tubes of 4 ml, one containing ethylenediamine tetraacetic (EDTA) and the other one heparin, immediately before, and 3, 30, 60, and 120 min after the end of the exercise bout. Blood was immediately centrifuged (10 min, 3000 rpm, 4°C) and plasma aliquots stored at −80°C until analyses.

2.3. Combined exercise training programme

The exercise training programme of the ACTIBATE study has been described in detail elsewhere (Sanchez-Delgado et al., 2015). Shortly, it consisted of a 24-week exercise programme with 150 min/week of endurance exercise and ∼80 min/week of resistance exercise. Participants (35M/75F) were randomised into three groups (control, moderate-intensity and vigorous-intensity). The control group did not perform any exercise. For the endurance exercise, the vigorous-intensity group performed 75 min/week at 60% of heart rate reserve (HRres) and 75 min/week at 80% HRres, whereas the moderate-intensity group performed all endurance training at 60% HRres. Concerning strength training, both groups completed 2 sessions/week consisting of exercises localised in the major muscle groups. Participants of the moderate-intensity group trained at 50% of 1-RM while the vigorous-intensity group at 70% 1-RM. Blood samples were collected in Vacutainer Tubes® 1–3 weeks before and 4–5 days after the training programme, after an overnight fast and avoiding physical exercise during the previous 48 h for vigorous-intensity and 24 h for moderate-intensity. Blood was immediately centrifuged (10 min, 3000 rpm, 4°C), and serum and plasma aliquots stored at −80°C until analyses.

2.4. Exerkines

All exerkines were determined using plasma samples containing EDTA except for meteorin-like, that was determine in plasma samples containing heparin. BAIBA was determined by high-performance liquid chromatography (HPLC), according to the previously described methodology (Molfino et al., 2017), by Agilent Liquid Chromatography System series 1100 (Agilent Technologies, USA) using columns from Phenomenex Luna HILIC (100 × 30 mm, Phenomenex, CA, USA). The kits used for the determination of the other exerkines plasma levels are pointed in Supplementary Table 1.

2.5. Body composition

Body weight and height were measured using a SECA scale and stadiometer (model 799, Electronic Column Scale, Hamburg, Germany). Lean mass, fat mass, and visceral adipose tissue (VAT) mass were assessed by dual-energy x-ray absorptiometry (HOLOGIC, Discovery Wi, Marlborough, MA). Waist circumference was measured twice with an elastic-plastic tape, and the mean value was obtained. These measures were obtained in overnight fasting conditions before the 24-week exercise programme.

2.6. Cardiometabolic risk factors

Serum glucose, total cholesterol, high-density lipoprotein-cholesterol (HDL-C), and triacylglycerols were quantified using an AU5832 automated analyser (Beckman Coulter Inc., Brea CA, USA). Low-density lipoprotein-cholesterol (LDL-C) was estimated as [total cholesterol – HDL-C – (triacylglycerols /5)]. Serum insulin was determined by the Access Ultrasensitive Insulin Chemiluminescent Immunoassay Kit (Beckman Coulter Inc., Brea, CA, USA) and homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as [insulin(μU/mL) x glucose (mmol/L)/22.5].

Systolic and diastolic blood pressure were measured twice on three different days by an automatic sphygmomanometer Omrom M2 (Omron Healthcare, Kyoto, Japan), and the mean was used for the analyses.

2.7. Physical fitness

To determine muscular strength, the 1-RM of the bench press and leg press were assessed using the Wathen equation (Baechle & Earle, 1994). We also measured handgrip strength using a Takei 5401 digital Grip-D hand dynamometer (Takei, Tokyo, Japan), by calculating

the mean between the best attempt performed with each hand. Maximal oxygen consumption (VO_2peak) was determined as the highest observed VO_2 during the maximum effort test, after removing obvious outliers. The time until exhaustion during the maximum effort test was also calculated.

2.8. Brown adipose tissue

BAT volume, ^{18}F-Fluoruodeoxyglucose (^{18}F-FDG) uptake, and mean radiodensity were assessed after a 2 h personalised cold exposure by a static ^{18}F-FDG positron emission tomography/computed tomography (PET/CT) scan as previously described (Martinez-Tellez et al., 2017). We used the standardised uptake value (SUV) threshold proposed in BARCIST 1.0 recommendations, and a fixed radiodensity range of −190 to −10 Hounsfield units.

2.9. Statistical analyses

Descriptive data are presented as mean and standard deviation, unless otherwise stated. Data out of the range of detection of the kits were excluded. A *P*-value <0.05 was considered statistically significant. Some of the variables did not follow a normal distribution (Shapiro–Wilk test <0.05), thus they were log2 transformed.

In the *discovery study*, the acute effect of exercise on the levels of exerkines measured before and 3, 30, 60 and 120 min after exercise was analysed by repeated measures analyses of variance (ANOVA). In the *confirmatory study*, paired student t-tests were employed instead. Finally, in the *chronic study*, the chronic effect of exercise on the levels of exerkines was analysed by 2-factor (*Group x Time*) ANOVAs. We also conducted analyses of covariance (ANCOVA) to compare the exercise training-induced changes (i.e. post-intervention – baseline) adjusted for the baseline level of each exerkine. Moreover, we analysed the *Sex x Time* interaction in the *confirmatory study* and *Sex x Time x Group* interaction effect in the *chronic study*. No significant interactions effects were detected, and thus the analyses were conducted combining men and women. We also explored the sex effect in the three different studies, without finding any statistical significance. Irisin was only detectable in 4 participants and pro-ANP and myostatin in 1 participant (data not shown), so no further analyses were performed.

We used data from the *confirmatory study* to calculate the acute change (Δ) in circulating concentrations of the exerkines, by subtracting the pre-exercise value to the selected post-exercise value (3 min for norepinephrine, lactate, BDNF, IL6 and FSTL1; 30 min for musclin and leptin; and 60 min for FGF21). We then analysed, by simple linear regression (Model I), the association between the Δ in exerkines concentrations and BAT volume, SUVmean, SUVpeak and mean radiodensity. These associations were also tested in multiple linear regression adjusting for the PET/CT scan date (Model II), BMI (Model III), and the baseline exerkines concentrations (Model IV). Similarly, we explored the association of the exercise-induced Δ in exerkines concentrations with body composition, cardiometabolic risk factors and physical fitness, by using simple linear regressions.

3. Results

3.1. Acute effect of endurance and resistance exercise on the circulating concentrations of exerkines

Endurance exercise increased the circulating concentration of BAIBA (5.3 vs 6.0 ppb, $P = 0.046$), lactate (0.9 vs. 5.5 mmol/L, $P < 0.001$), norepinephrine (1.8 vs. 3.5 ng/mL, $P = 0.004$), BDNF (2.5 vs. 4.8 ng/mL, $P = 0.008$), IL6 (4.5 vs. 6.9 pg/mL, $P = 0.052$), and FSTL1 (13.9 vs. 16.7 ng/ml, $P = 0.042$) immediately after exercise (Figure 1). Musclin (254.3 vs. 319.1 pg/mL, $P = 0.076$) and FGF21 (23.5 vs 72.5 pg/mL, $P = 0.066$) were also upregulated by exercise, reaching a peak 30 and 60 min after exercise, respectively (Figure 1). In contrast, circulating leptin was reduced after the endurance exercise bout ($P = 0.025$), with the strongest effect appearing 30 min after the exercise (6.6 vs 5.9 µg/L) (Figure 1). The other studied exerkines were not modified by endurance exercise (Figure 1). Those exerkines whose concentrations were modified by endurance exercise were studied in a wider cohort for the *confirmatory study* ($n = 38$) at the time they were more severely modified except for BAIBA, since it was detected only in 60% of the samples. The results observed in the *discovery study* were replicated in the *confirmatory study* (Supplementary Figure 2).

Regarding the acute resistance exercise, we observed an increase of lactate levels (0.8 vs. 3.1 mmol/L, $P < 0.001$) immediately after exercise (Figure 2). The rest of circulating exerkines were not modified by resistance exercise (Figure 2).

3.2. Chronic effect of exercise on circulating exerkines

The resting and fasting concentrations of all analysed exerkines were not modified by the 24-week exercise

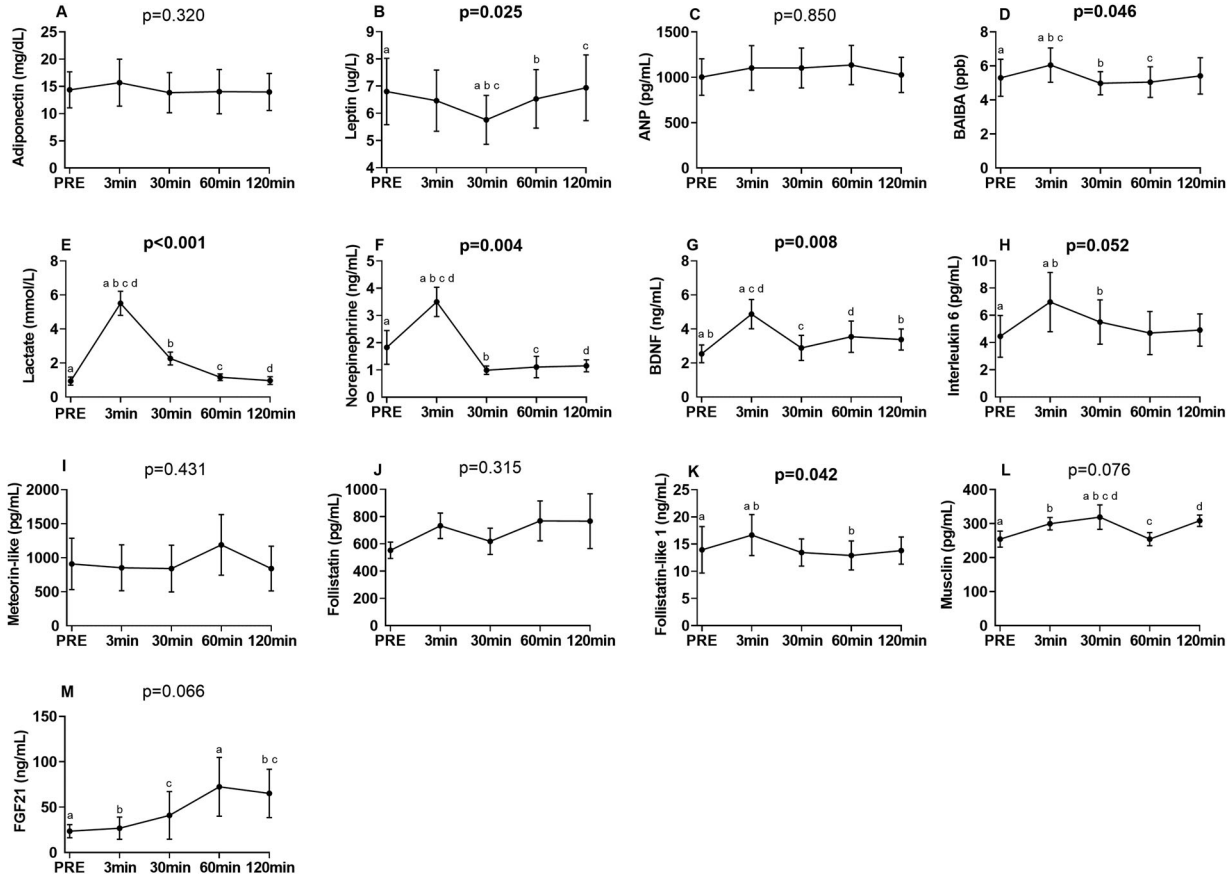

Figure 1. Acute effect of endurance exercise on circulating concentrations of endocrine signals able to regulate brown adipose tissue metabolism and/or white adipose tissue browning in the *discovery study* ($n = 10$). Circulating plasma concentrations of adiponectin (A), leptin (B), ANP (C), BAIBA (D), lactate (E), norepinephrine (F), BDNF (G), IL6 (H), meteorin-like (I), follistatin (J), follistatin-like 1 (K), musclin (L), FGF21 (M) were measured before and 3, 30, 60 and 120 min after an incremental maximum effort test. P from repeated measured analysis of variance. Error bars indicate standard error. Common letters indicate significant differences in post-hoc analyses. Abbreviations: ANP: atrial natriuretic factor; BAIBA: β-aminoisobutyric acid; BDNF: brain-derived neurotrophic factor; FGF21: fibroblast growth factor 21.

training programme (Figure 3). Similar results were also obtained when performing analyses of covariance (ANCOVA) (data not shown).

3.3. Association of the endurance exercise-induced changes in exerkines with BAT related variables, body composition, cardiometabolic risk factors and physical fitness

Supplementary Table 2 shows the association between the endurance exercise-induced acute changes in circulating exerkines and BAT volume, ^{18}F-FDG uptake and mean radiodensity. The endurance exercise-induced change in lactate concentration was positively associated with BAT radiodensity ($\beta = 1.050$, $R^2 = 0.258$, $P = 0.011$) and these results persisted after adjusting for the PET/CT scan date, BMI, and the baseline lactate concentration (all $P \leq 0.015$). When adjusting the analyses for PET/CT scan date, lactate concentration was also positively associated with BAT SUVpeak ($\beta = 0.604$; $R^2 = 0.538$; $P = 0.033$) (Supplementary Table 2). Moreover, when lactate concentration was log2 transformed we also observed associations with BAT volume ($\beta = 25.857$; $R^2 = 0.196$; $P = 0.011$), BAT SUVmean ($\beta = 1.017$; $R^2 = 0.169$; $P = 0.019$), BAT SUVpeak ($\beta = 17.395$; $R^2 = 0.210$; $P = 0.008$), and BAT radiodensity ($\beta = 19.808$; $R^2 = 0.168$; $P = 0.047$) (Supplementary Figure 3). The other studied exerkines were not associated with BAT related variables (Supplementary Table 2).

Supplementary Table 3 shows the associations of endurance exercise-induced changes in circulating concentrations of exerkines with body composition, cardiometabolic risk factors, and physical fitness. The exercise-induced decrease in leptin concentration was negatively associated with BMI ($\beta = -1.441$, $R^2 = 0.189$, $P = 0.011$), fat mass ($\beta = -3.882$, $R^2 = 0.274$, $P = 0.002$), VAT mass ($\beta = -57.063$, $R^2 = 0.147$, $P = 0.028$), triacylglycerols ($\beta =$

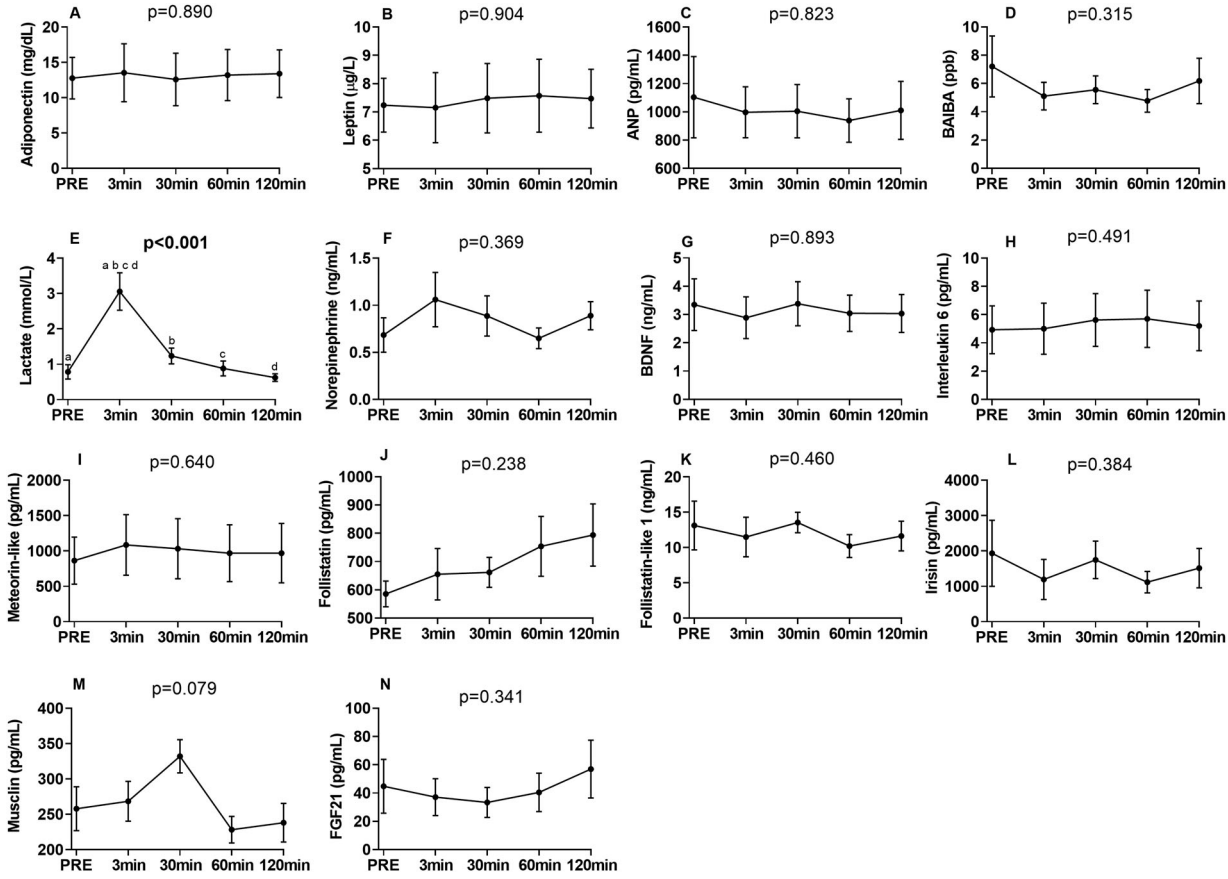

Figure 2. Acute effect of resistance exercise on circulating concentrations of endocrine signals able to regulate brown adipose tissue metabolism and/or white adipose tissue browning in the *discovery study* ($n = 10$). Circulating plasma concentrations of adiponectin (A), leptin (B), ANP (C), BAIBA (D), lactate (E), norepinephrine (F), BDNF (G), IL6 (H), meteorin-like (I), follistatin (J), follistatin-like 1 (K), irisin (L), musclin (M) and FGF21 (N) were measured before and 3, 30, 60 and 120 min after a session of resistance exercise. P from a repeated measured analysis of variance. Error bars indicate standard error. Common letters indicate significant differences in post-hoc analyses. Abbreviations: ANP: atrial natriuretic factor; BAIBA: β-aminoisobutyric acid; BDNF: brain-derived neurotrophic factor; FGF21: fibroblast growth factor 21.

−20.881, $R^2 = 0.115$, $P = 0.040$) and diastolic blood pressure ($\beta = -2.831$, $R^2 = 0.153$, $P = 0.020$). However, these associations disappeared after adjusting for baseline leptin concentrations. The log2 exercise-induced increase in BDNF concentration was also negatively associated with BMI ($\beta = -5.426$, $R^2 = 0.178$, $P = 0.018$), HOMA-IR ($\beta = -1.559$, $R^2 = 0.244$, $P = 0.003$), insulin ($\beta = -6.263$, $R^2 = 0.229$, $P = 0.004$), glucose ($\beta = -9.513$, $R^2 = 0.267$, $P = 0.001$), waist circumference ($\beta = -14.990$, $R^2 = 0.161$, $P = 0.025$), and fat mass ($\beta = -13.256$ $R^2 = 0.226$, $P = 0.007$) (data not shown). These associations persisted after adjusting for baseline plasma BDNF concentration. None of the exercise-induced changes in the concentration of the other exerkines was associated with body composition parameters, cardiometabolic risk factors or physical fitness (Supplementary Table 3). The association among the exercise-induced changes in circulating exerkines assessed in the *confirmatory study* is shown in Supplementary Table 4.

4. Discussion

In the present study, we investigated, in young sedentary adults, the acute and chronic effect of exercise on plasma levels of 16 exerkines that have been shown to regulate BAT metabolism and/or WAT browning in preclinical models. We found that a maximum walking effort test on a treadmill increased the circulating concentration of BAIBA, lactate, norepinephrine, BDNF, IL6, FSTL1, musclin, and FGF21, reduced leptin concentration, and did not modify the levels of adiponectin, ANP, meteorin-like and follistatin. Plasma levels of pro-ANP, myostatin, and irisin were not detected in more than half of the individuals. Interestingly, the exercise-induced change in lactate concentration was positively associated with BAT volume, ^{18}F-FDG uptake and radiodensity. A bout of resistance exercise increased lactate levels, without affecting the rest of analysed exerkines. Finally, we did not observe any long-term effect of an exercise training programme including endurance and

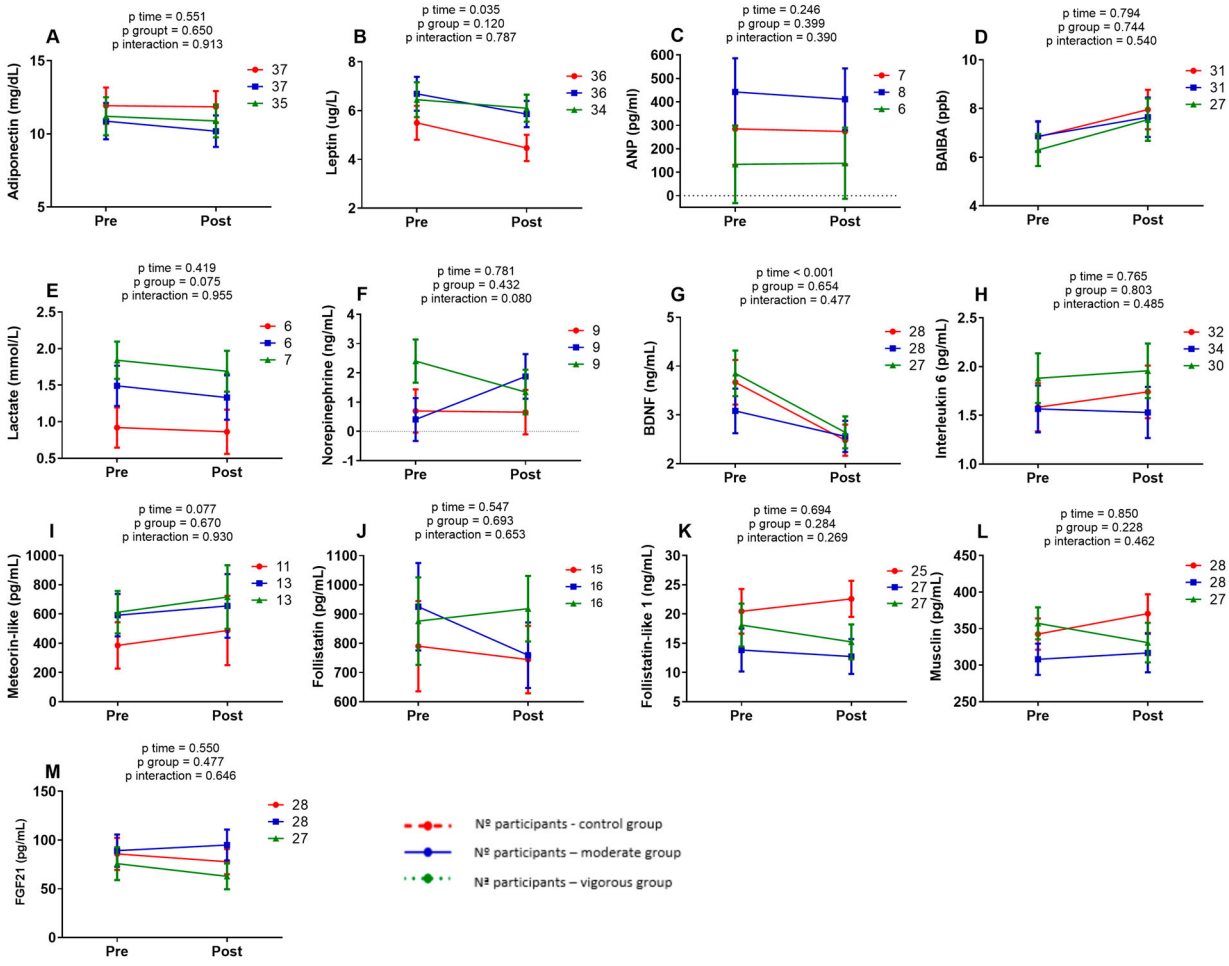

Figure 3. Chronic effect of a 24-week combined exercise (endurance + resistance) training programme on fasting circulating plasma concentrations of endocrine signals able to regulate brown adipose tissue metabolism and/or white adipose tissue browning in the *chronic study*. Circulating plasma concentrations of adiponectin (A), leptin (B), ANP (C), BAIBA (D), lactate (E), norepinephrine (F), BDNF (G), IL6 (H), meteorin-like (I), follistatin (J), follistatin-like 1 (K), musclin (L) and FGF21 (M) were determined 1–3 weeks before and 4–5 days after the training programme. *P* values from 2-factor (*Group* x *Time*) analyses of variance. Circulating levels of pro-ANP, irisin, and myostatin were detected in less than 50% of sample size (data not shown). Error bars indicate standard error. Abbreviations: ANP: atrial natriuretic factor; BAIBA: β-aminoisobutyric acid; BDNF: brain-derived neurotrophic factor; FGF21: fibroblast growth factor 21.

resistance exercise on the plasma levels of the studied exerkines.

The changes observed after acute endurance exercise in the circulating concentrations of leptin, lactate, norepinephrine, BDNF, IL6, FSTL1, and FGF21, are consistent with previous studies' results (Mendez-Gutierrez et al., 2020). However, this is the first study reporting an increase in plasma levels of musclin after a bout of exercise in humans. Stautemas et al. previously reported an increase in BAIBA plasma levels after 1 h of an incremental cycling test in trained subjects (Stautemas et al., 2019), whereas Morales et al did not observe any effect in untrained individuals (Morales et al., 2017). Although we found an increase in plasma BAIBA levels in our *discovery study*, it should be noted that BAIBA concentrations were detected only in 60% of the participants. The differences observed between our study and others might be explained by the different training status of participants, as well as the duration and intensity of exercise. In our study, untrained individuals performed a short but very intense session of exercise.

In our study, a maximum effort test on a treadmill did not alter the plasma levels of adiponectin, ANP, meteorin-like or follistatin, which have been shown to produce WAT browning in preclinical models (Mendez-Gutierrez et al., 2020). Previous studies in middle-aged and young adults observed increases in plasma levels of ANP (Peres et al., 2018), follistatin (He et al., 2019), and meteorin-like (Saghebjoo, Einaloo, Mogharnasi, & Ahmadabadi, 2018) after a session of moderate-intensity endurance exercise between 40 and 60 min of duration. These findings suggest that a lower intensity and greater duration might be required to stimulate these exerkines secretion. Moreover, increases in follistatin

concentration have been reported after 3 h of exercise (He et al., 2019), whereas the last blood sample in our study was collected 2 h after the end of the exercise bout. Previous studies have also reported controversial results for the acute effect of endurance exercise on circulating adiponectin (Jürimäe et al., 2006; Kraemer et al., 2003), myostatin (He et al., 2019), and irisin (He et al., 2019; Mendez-Gutierrez et al., 2020). Furthermore, irisin results should be considered with caution since the detectability of irisin in humans has been questioned (Albrecht et al., 2015). Overall, exercise characteristics such as intensity or duration, or characteristics of the study subject, such as age or training status, are likely explaining part of these controversies. It also should be noted that the exercise-induced changes exhibited different kinetics for some of the analysed exerkines. This may be due to the diverse nature of the molecules. While some of them are end products of metabolic pathways, such as lactate, others, like FGF21, are proteins resulting from the complex processes of transcription and translation, which indeed takes time.

We observed an intriguing and consistent positive association between the exercise-induced change in lactate concentrations and BAT volume, ^{18}F-FDG uptake and radiodensity. Lactate is a product of anaerobic glycolysis, whose secretion is related with the exercise intensity and type IIb muscle fibre proportion (Schranner, Kastenmüller, Schönfelder, Römisch-Margl, & Wackerhage, 2020). Murine brown adipocytes internalise lactate in response to exercise, leading to an increase in UCP1 thermogenic activity and UCP1 and FGF21 expression in adipocytes, inducing WAT browning (Carrière et al., 2020). Thus, the association found in our study might reflect the existence of a muscle-BAT crosstalk taking place during exercise. Indeed, other molecules have been previously shown to be part of this bidirectional inter-organ communication (Kong et al., 2018; Stanford et al., 2018). The relation between BAT function and skeletal muscle composition and function deserves further investigation.

We found a negative association between the acute endurance exercise-induced change in BDNF plasma levels and BMI, HOMA-IR, glucose, insulin, waist circumference, and fat mass. It suggests that participants with a fatter and less favourable metabolic profile have a smaller increase in BDNF after endurance exercise than their counterparts. These results are in line with those obtained by Roth et al (Roth, Elfers, Gebhardt, Müller, & Reinehr, 2013), who observed unchanged serum BDNF levels after one year of exercise intervention in children with obesity, but not in children with normal-weight. Other authors have reported negative associations between serum BDNF levels and BMI (Corripio et al., 2012), which is in line with our results. We also observed a negatively association between exercise-induced decrease in leptin concentration and BMI, fat mass, VAT mass, triacylglycerols and diastolic blood pressure. However, these associations disappeared after adjusting for baseline leptin concentrations, suggesting that these parameters are associated with baseline leptin plasma levels instead of with the exercise-induced change (Aguilera, Gil-Campos, Cañete, & Gil, 2008).

We also explored the effect of resistance exercise on the selected exerkines. We only observed an increase of plasma levels of lactate after the bout of resistance exercise, which is in line with results previously observed (Schranner et al., 2020). It should be remarked that the increase observed in plasma levels of lactate after the maximum effort test was twice the observed in the session of acute resistance exercise, probably due to the difference in intensity (2.2 mmol/L vs. 4.6 mmol/L, $P = 0.021$, Supplementary Figure 4). Previous studies also failed to observe any effect of resistance exercise on circulating concentrations of ANP (MacDonald et al., 1999), myostatin (Han, Hsiao, Wang, Chen, & Yang, 2016), FGF21 (Parmar et al., 2018), and BDNF (Goekint et al., 2010). Other exerkines, such as meteorin-like, musclin, FSTL1, adiponectin, and BAIBA, which we found not to be modified, have not been analysed in humans in response to resistance exercise. On the other hand, other studies have reported increases in norepinephrine (Kraemer et al., 2015), IL6 (Parmar et al., 2018), and decreases in leptin concentrations (Zafeiridis, Smilios, Considine, & Tokmakidis, 2003) immediately and 30 min after resistance exercise. Importantly, the lack of effect observed in our study is likely explained, at least in part, by the training load imposed by the experimental session. The resistance exercise session was designed with the primary aim of assessing muscle strength, and thus, it was characterised by large recovery periods and relatively low training volume. New studies with more intense and denser resistance exercise sessions are needed to explore the effect of resistance exercise on these exerkines in humans.

Finally, we did not observe any effect of the 24-week training programme on the resting circulating concentrations of exerkines. Some studies have reported an increase in circulating concentrations of some exerkines, such as adiponectin (Racil et al., 2013), BDNF (Pereira et al., 2013), FGF21 (Keihanian, Arazi, & Kargarfard, 2019), follistatin (Bagheri et al., 2020), and a decrease in myostatin (Bagheri et al., 2020) and leptin concentrations (Zaccaria, Ermolao, Brugin, & Bergamin, 2013). On the one hand, Racil et al observed an increase in plasma adiponectin levels in adolescent girls with

obesity after 12 weeks of interval training (Racil et al., 2013). Similarly, Pereira et al reported an increase in plasma BDNF levels in elderly women after 10 weeks of strength-training programme (Pereira et al., 2013). Regarding FGF21, Keihanian et al. also observed an increase in serum levels in males with type-2 diabetes after a 8-week training programme (both resistance and aerobic exercise) (Keihanian et al., 2019). In addition, Bagheri et al. studied a 8-week training programme combining resistance and aerobic exercise in 30 sarcopenic elderly men (Bagheri et al., 2020), observing an increase in serum follistatin levels and a decrease in serum myostatin levels. Finally, other studies have reported a decrease in plasma and serum leptin levels after chronic exercise in overweight and obese individuals, although it seems to be fat mass dependent (Zaccaria et al., 2013). The differences with the results obtained in our study might be explained by the health status and age of the individuals as well as the intensity and the duration of the training programme.

The findings of this study should be considered with caution, as some limitations are present. The importance of including sex as a biological variable is widely recognised and it is plausible that the effect of exercise on BAT-related exerkines differs in men and women (Collado-Boira et al., 2021; Lee, 2018). However, our study was not designed for studying the effect of sex, and lack the statistical power to adequately tested sex differences. In addition, participants did not follow a familiarisation period before the muscle strength measurements. The endurance and resistance sessions consist of relatively low training load and thereby, the stimulus could have not been strong enough to trigger a significant release of the aforementioned exerkines to the bloodstream. Furthermore, despite the ^{18}F-FDG PET/CT is currently the best available method to assess BAT volume, it presents serious limitations for assessing its thermogenic activity (Carpentier et al., 2018).

In conclusion, we found that a short and intense endurance exercise bout increases blood concentrations of norepinephrine, lactate, BDNF, IL6, FSTL1, musclin, and FGF21, whereas it decreases leptin concentration. In contrast, the concentration of adiponectin, ANP, meteorin-like and follistatin was not modified by the endurance exercise bout. A bout of resistance exercise only increased the plasma levels of lactate immediately after resistance exercise. On the other hand, neither a low-volume resistance exercise session nor a 24-week exercise training programme including both endurance and resistance training did modify the concentration of these endocrine signalling molecules. Altogether, our results show that a short and intense endurance exercise bout increases the plasma levels of several exerkines that could regulate BAT metabolism and WAT browning in sedentary young adults, yet only the exercise-induced change in lactate concentrations was associated with BAT volume, glucose uptake and radiodensity.

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