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Effect of a 24-Week Concurrent Exercise Intervention on Neck Adiposity and Its Distribution in Young Adults: The ACTIBATE Randomized Controlled Trial

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Neck adipose tissue (NAT) accumulation and neck circumference are independent predictors of cardiometabolic risk (CMR) and low-grade chronic inflammation in young adults. The present study examines whether a 24-week concurrent exercise intervention can reduce NAT volume and neck circumference in young adults, and whether any changes in these variables are related to changes in body composition, CMR, and the inflammatory profile. Seventy-four participants (51 women, age 22 ± 2 years) were included in the main analyses, after being randomly assigned to either a (a) control ($n = 34$), (b) moderate-intensity exercise ($n = 19$), or (c) vigorous-intensity exercise ($n = 21$) group. Participants in the exercise groups trained 3–4 days/week (endurance + resistance exercise training). NAT volume and NAT distribution across different depots were estimated using computed tomography before and after the intervention. Anthropometric variables, body composition (determined by dual-energy X-ray absorptiometry), and CMR/inflammatory markers were also recorded. The exercise intervention did not reduce the total NAT volume, nor was NAT distribution affected ($p > .05$). However, it did reduce neck circumference in the vigorous-intensity exercise group compared with the moderate-intensity exercise and control groups (by 0.8 and 1 cm, respectively, $p \leq .05$). Changes in total NAT and neck circumference were positively, albeit weakly, related (adj. R^2: .05–.21, all $p \leq .05$) to changes in body weight and adiposity, leptin (only total NAT), and CMR (only neck circumference). Altogether 24 weeks of concurrent exercise does not appear to reduce NAT accumulation in young adults, but may slightly reduce neck circumference in those who partake in vigorous exercise.

Keywords: aerobic exercise, cardiovascular disease, neck fat, upper body fat, strength exercise, volumetric assessment

The last decade has seen attention focused on previously overlooked adipose tissue depots that might influence cardiometabolic risk (CMR). Among these depots, recent evidence has centered on the neck adipose tissue (NAT), a heterogeneous and anatomically disperse depot within the nonsplanchnic upper body fat, which is divided in different compartments, and whose volume and distribution can be accurately quantified by using computed tomography (CT; Torriani et al., 2014) or magnetic resonance imaging (Cielo et al., 2021). NAT is positively related to total and central body fat (Arias-Tellez & Acosta, 2021; Torriani et al., 2014), to CMR independent of body mass index (BMI) or visceral adipose tissue (VAT) mass (Pandzic Jaksic et al., 2018; Rosenquist et al., 2014; Torriani et al., 2014), and it has been associated with all-cause mortality (Tal & Litovchik, 2019).

Physical activity appears to be one of the major factors affecting NAT accumulation and distribution. We recently showed that regular moderate intensity and/or overall physical activity are associated with lower intermuscular and total NAT in young men (Arias-Tellez et al., 2021), but the cross-sectional design of that study meant causality could not be confirmed. Exercise is, however, a cornerstone in the treatment of many diseases (Fiuza-Luces et al., 2013), and the evidence indicates that its cardiometabolic benefits are mediated by increases in muscle mass and strength as well as the reduction in whole-body adiposity and lipid deposition in ectopic depots (Hong et al., 2014; Maillard et al., 2018; Zhang et al., 2017). There is, therefore, a need to determine whether exercise reduces NAT accumulation, and therefore contributes toward reducing CMR and improving the inflammatory profile. Noteworthily, the inclusion of measurements such as neck circumference (a proxy of NAT accumulation) to predict CMR could be of high clinical interest, especially considering how easy it is to take (e.g., no need to remove clothing) or affected by food intake.

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The aims of the present work were (a) to examine the dose–response effect of a 24-week supervised exercise intervention on NAT volume and neck circumference in young adults and (b) to determine whether changes in NAT volume and neck circumference are related to changes in other body composition variables, CMR, and the inflammatory profile.

Methods

Study Design

The present work is an ancillary study of the Activating Brown Adipose Tissue Through Exercise in Young Adults (ACTIBATE) trial (ACTIBATE, ClinicalTrials.gov, ID: NCT02365129), a randomized controlled trial initially designed to investigate the dose–response effect of a 24-week concurrent exercise intervention on the mass and activity of brown adipose tissue (BAT) in young adults. The study was approved by the University of Granada Ethics Committee on Human Research (n° 924) and by that of the *Servicio Andaluz de Salud*. The study was performed in accordance with the Declaration of Helsinki (2013 revision). All participants gave their written, informed consent to be included. Participant recruitment, and all assessments and interventions were conducted at the Sport and Health Joint University Institute (iMUDS), and at the University Hospital "Virgen de las Nieves," both in Granada, Spain. The study was conducted over two consecutive years (from September 2015 to June 2016, and from September 2016 to June 2017). In both years, participants were enrolled in four different waves (16–24 participants in each wave) starting in September–December.

Study Participants

The study protocol, the description of procedures, and the inclusion–exclusion criteria are provided elsewhere (Sanchez-Delgado et al., 2015). Briefly, all participants examined were 18–25 years old, had a BMI of >18.5 kg/m^2, were sedentary (i.e., partaking in < 20 min moderate–vigorous physical activity <3 days/week at baseline), were nonsmokers, took no contraindicated medication, had a stable body weight over the previous 3 months (changes <3 kg), had no cardiometabolic disease (e.g., hypertension or diabetes), and had no first-degree relative who had suffered cancer. After holding the initial information meetings, 371 participants confirmed their interest in the project and were assessed for eligibility (see flowchart, Figure S1 in the Supplementary Material [available online]); 145 were finally enrolled.

Intervention

After baseline assessment of the primary and secondary outcome variables (see below), participants were randomly assigned to either (a) a control group (CON, no exercise, $n = 54$), (b) a moderate-intensity exercise group (MOD-EX, $n = 48$), or a (c) vigorous-intensity exercise group (VIG-EX, $n = 43$). The characteristics of the groups are explained later. Participants assigned to the control group were instructed to maintain their habitual lifestyle (i.e., similar physical activity levels and diet).

Supervised Concurrent Exercise Intervention

A detailed description of the exercise intervention can be found in the Supplementary Material (available online) as well as elsewhere (Sanchez-Delgado et al., 2015). Briefly, it combined both endurance and resistance training and was always supervised by a researcher specialized in the field of sports science. The design of the exercise program was based on the physical activity recommendations for adults proposed by the World Health Organization (2010).

a. *Frequency*. Participants came to the research center for training for 24 weeks, 3–4 days/week. Endurance training was performed in all sessions, whereas resistance training was performed in two of these weekly sessions.

b. *Volume and intensity*. Both the MOD-EX and VIG-EX groups performed 150 min/week of aerobic exercise and ≈ 80 min/week of strength training.

- For endurance exercise, the MOD-EX group trained for a total of 150 min/week of aerobic exercise at 60% HR_{res} (heart rate reserve), whereas the VIG-EX group performed 75 min/week at moderate intensity (i.e., 60% HR_{res}) and 75 min/week at vigorous intensity (i.e., 80% HR_{res}). HR_{res} was not reassessed through the intervention.
- The resistance training was performed at 50% of the 1 repetition maximum (RM) for the MOD-EX group and at 70% RM for the VIG-EX group. RM was reassessed every 5 weeks to ensure an efficient resistance training progression.

c. *Types of exercise*. Endurance training was performed using a treadmill, static bike, or elliptical bike (in blocks of 10 min with a short break between them), at the prespecified intensity. Resistance training mainly comprised exercises focused on the upper and lower body major muscle groups (e.g., bench press, leg press, etc.), as well as compensatory exercisers (e.g., core stability).

The intensity of the endurance training for the VIG-EX group was progressively increased (familiarization period) until the target intensity was reached (Sanchez-Delgado et al., 2015). All participants wore an RS800CX exercise heart rate monitor (Polar Electro Öy) during the exercise sessions in order to monitor exercise intensity.

A maximum of 16 people (and at least one expert supervisor) were allowed in each session. In special circumstances (holidays or absence from training sessions that could not be retaken at the research center), participants were provided with a pulsometer, an elastic band and specific instructions to carry out adapted training sessions at home. Participants could contact the research staff regarding any doubt or problem.

Primary Outcomes

^{18}F-FDG-PET/CT Acquisition and Analysis

^{18}F-fluordeoxyglucose (^{18}F-FDG) positron emission tomography and CT scanning were performed using a Biograph 16 positron emission tomography combined with computed tomography (PET-CT) scanner (Siemens) from the atlas vertebra to the midchest region (Martinez-Tellez et al., 2022). The original aim of the ACTIBATE study (Sanchez-Delgado et al., 2015) was to detect the ^{18}F-FDG uptake of BAT. Consequently, the participants were submitted to a 60-min personalized cooling protocol prior to the injection of a bolus of ^{18}F-FDG with the objective of stimulating BAT glucose uptake (Martinez-Tellez et al., 2017). After 1 hr, with the participants lied in supine position and with a thin pillow below their heads, a low-dose CT (120 k) scan was performed for attenuation correction and anatomic localization.

i. Neck Adipose Tissue Quantification and Neck Circumference. For the quantification of NAT, only the CT images of the PET/CT scan was analyzed (by a single researcher) using the Beth Israel plugin for FIJI software https://sourceforge.net/projects/bifijiplugins/. Using a three-dimensional axial technique, several regions of interest (ROIs) were outlined at the level of C5 to determine the NAT volume and the distribution of fat across the different NAT compartments (see a, b, and c below). The NAT volumes in these ROIs were calculated by determining the number of voxels within the radiodensity range of −300 to −10 Hounsfield Units.

a. Subcutaneous NAT: Adipose tissue in the posterior neck, between the skin and deep cervical fascia.

b. Intermuscular NAT: Adipose tissue between the sternocleidomastoid, levator scapulae, semispinalis, and trapezius muscles separated from the subcutaneous fat by the deep cervical fascia. No overlapping was allowed between the subcutaneous NAT and this compartment.

c. Perivertebral NAT: Adipose tissue interspersed between the muscles surrounding vertebra C5.

Another ROI was also drawn at the level of C5, and voxels in the radiodensity ranges −300 and −10 Hounsfield Units and 9 to 150 Hounsfield Units were used to determine the total NAT and neck lean volumes, respectively (the latter including skeletal muscle tissue, blood vessels, and some internal organs; Chung et al., 2009). A detailed description of the analytical procedures is reported elsewhere (Arias-Tellez & Acosta, 2021). The percentage of total NAT within this ROI was also calculated (Arias-Tellez & Acosta, 2021).

Neck circumference was measured perpendicular to the longitudinal axis of the neck using an inextensible metallic tape running over the thyroid cartilage (Stewart et al., 2011). The participants were in anatomical position, standing or sitting with the head in the Frankfurt plane and the shoulders relaxed. The relative technical error of measurement (for test–retest of neck circumference measurements taken before and after the intervention) was ≤1% (a sign of a skillful anthropometrist; Norton & Olds, 2000).

Secondary Outcomes

Anthropometry and body composition, cardiometabolic and inflammatory markers, and energy and macronutrient intake variables measurements were carried out as detailed in Appendix A (see Supplementary Material [available online]).

Sample Size

This was a secondary study. Taking this, and the exploratory nature of the work (the literature contains no other study that investigates the effects of exercise on NAT volume and distribution) into account, formal calculations of statistical power for detecting differences could not be performed.

Randomization and Blinding

Eligible participants were randomly assigned to the control or exercise groups (after completing all baseline assessments) using a simple, unrestricted randomization program (Schulz & Grimes, 2002). The principal investigator (Ruiz) was the only person aware of the participants' group assignments; this information was only communicated to the rest of the research team once an eligible participant was ready to start the intervention phase.

Statistical Analysis

The characteristics of the study participants were recorded using descriptive statistics (Table 1). Mean and *SD* or median values and interquartile ranges (percentile 25–75) are provided for normally and nonnormally distributed variables, respectively. Differences among the groups at baseline were examined by one-way analysis of variance for normally distributed variables and Kruskal–Wallis tests for nonnormally distributed variables. The influence of the interaction *exercise group* × *participant sex* on neck measurements was checked by linear regression. Given the reduced sample size, all analyses were performed pooling women and men together. Important confounders (according to existing evidence), and/or which were statistically related to the outcomes (e.g., the baseline value of each respective outcome and sex), were included in the regression models.

To address the effect of the exercise program on NAT volume and neck circumference, and considering that most variables were not normally distributed, bootstrapping analysis of variance was used to analyze the dose–response effect with no adjustments (Model 0; Figure 1). Bootstraps for pairwise comparisons were used to detect differences among groups. Mean differences and 95% bias-corrected-and-accelerated confidence intervals (based on 1,000 bootstrap samples) were recorded. These analyses were replicated including the baseline values of the respective outcome, or the baseline value of the respective outcome plus sex, as potential confounders (Models 1 and 2).

Only those exercisers who attended more than 70% of the total training sessions and who adhered to the training intensity established were considered for the main (per-protocol) intervention analyses (Figure 1). Adherence to the set training intensity was considered as spending ≥50% of the total training time at moderate intensity (55%–65% HR_{res}) and <20% at transition intensity (65%–75% HR_{res}) for the MOD-EX group, and as >40% of the total training time at vigorous intensity (>75% HR_{res}) for the VIG-EX group.

Interquartile regressions were performed to examine the relationship of the change in NAT volume and neck circumference (Table 2), neck lean volume (Table S1 in the Supplementary Material [available online]), and VAT mass (as a reference tissue, Table S2 in the Supplementary Material [available online]) with the change in other body composition variables, CMR, and the inflammatory profile. These analyses were adjusted for sex and intervention group. Participants attending ≥70% of the total exercise training sessions were included in these analyses.

Nonstandardized β coefficient (*B*), standard deviation (*SE*), adjusted *R*-squared (R^2), and *p* values are reported. No intention-to-treat analyses were performed. All analyses were conducted using SPSS software (version 26.0), except for the interquartile regressions, which were performed using STATA software (StataCorp). Significance was set at $p \leq .05$.

Results

As shown in Figure S1 (flowchart) (see Supplementary Material [available online]), of the 194 participants initially enrolled in the exercise intervention, 145 were randomized to the CON ($n = 54$), MOD-EX ($n = 48$), or VIG-EX ($n = 43$) groups. However, only 120 of these participants underwent the ^{18}F-FDG-PET-CT scan to

Table 1 Descriptive Characteristics of the Study Participants

	Preintervention						Postintervention						
	CON		MOD-EX		VIG-EX		CON		MOD-EX		VIG-EX		
		N		*N*		*N*		*N*		*N*		*N*	*p*
Age (years)	22 (18–26)	*34*	22 (19–26)	*19*	23 (19–27)	*21*							
Sex, female, *n* (%)	21 (61.8)	*34*	13 (68.4)	*19*	17 (81)	*21*	21 (61.8)	*34*	13 (68.4)	*19*	17 (81)	*21*	
Anthropometry and body composition													
Weight (kg)	67.2 (56.2–77)	*34*	69.1 (15.8)	*19*	69.9 (13.4)	*21*	69.5 (16.8)	*34*	68.8 (15.4)	*19*	69 (11.6)	*21*	.87
Height (m)	1.68 (0.09)	*34*	1.67 (0.08)	*19*	1.67 (0.08)	*21*	1.67 (1.6–1.75)	*34*	1.68 (0.08)	*19*	1.68 (0.08)	*21*	.94
Body mass index (kg/m^2)	23.3 (21–26.5)	*34*	23.1 (20.5–27.3)	*19*	24.9 (3.6)	*21*	24.3 (4.4)	*34*	23.1 (20.7–26.9)	*19*	24.5 (3)	*21*	.50
Waist circumference (cm)	79.4 (69–90.5)	*34*	80.3 (14.4)	*19*	79.9 (11.8)	*20*	82.5 (13.9)	*34*	76.2 (68.0–84.8)	*19*	80.5 (10.1)	*21*	.98
Lean mass (kg)	38.4 (31.9–52.1)	*34*	41.3 (8.6)	*19*	37.1 (33.7–47.5)	*21*	39.2 (32.6–54)	*34*	43 (9)	*19*	39.6 (36.2–49.7)	*21*	.88
Fat mass (kg)	23.3 (16.7–30.9)	*34*	19.5 (16.2–31)	*19*	25.6 (6.6)	*21*	20.9 (17.5–26.6)	*34*	22.2 (8.8)	*19*	22.8 (5.3)	*21*	.44
Fat mass (%)	34.4 (7.5)	*34*	34.9 (8.3)	*19*	37.5 (6.1)	*21*	33.6 (7.3)	*34*	32.3 (7.4)	*19*	33.9 (6.2)	*21*	.30
VAT mass (g)	310 (162–453)	*34*	327 (195)	*19*	315 (236–502)	*21*	292.3 (160–383)	*34*	228.2 (174–365)	*19*	287 (137)	*21*	.66
Neck measures													
Subcutaneous NAT volume (ml)	13.2 (6.7–27.1)	*23*	12.4 (8.2–31.0)	*16*	16.6 (10.7)	*16*	12.7 (6.1–25)	*23*	11.1 (6.1–18.9)	*16*	16.5 (13)	*16*	.74
Intermuscular NAT volume (ml)	0.7 (0.3–1.3)	*23*	1.1 (0.6–2.2)	*16*	0.9 (0.8)	*16*	0.7 (0.25–1.2)	*23*	0.7 (0.3–1.8)	*16*	1.2 (0.3)	*16*	.28
Perivertebral NAT volume (ml)	0.2 (0.1–0.3)	*23*	0.2 (0.19–0.48)	*16*	0.3 (0.3)	*16*	0.3 (0.2)	*23*	0.2 (0.1–0.3)	*16*	0.3 (0.1)	*16*	.46
Total NAT volume (ml)	9.5 (5.0–19.6)	*26*	8.6 (5.1–20.9)	*16*	11.8 (5.1–20.5)	*17*	9.8 (4.8–19.2)	*26*	7.3 (4.9–19.5)	*16*	10.7 (5.6–26.0)	*17*	.97
Neck lean volume (ml)	105.3 (35.2)	*26*	111.7 (31.3)	*16*	100.4 (28.7)	*17*	111.8 (39.7)	*26*	113.0 (33.6)	*16*	99.4 (25.5)	*17*	.61
Neck circumference (cm)	34.3 (4.2)	*21*	33 (30.6–35.7)	*13*	33.4 (3.2)	*11*	34.6 (3.8)	*21*	33.7 (3.5)	*13*	32.6 (3)	*11*	.83

Note. Continuous variables are presented as means (*SD*s) when normally distributed, or medians (interquartile range) when not, unless otherwise indicated. The sample size is provided on the right side (letters in italics). One-way ANOVA for normally distributed variables and the Kruskal–Wallis test for nonnormally distributed variables were used to compare anthropometry, body composition, and neck measures among the intervention groups at baseline. The *p* value is provided for these comparisons. ANOVA = analysis of variance; CON = control group; NAT = neck adipose tissue; MOD-EX = moderate-intensity exercise group; VAT = visceral adipose tissue; VIG-EX = vigorous-intensity exercise group.

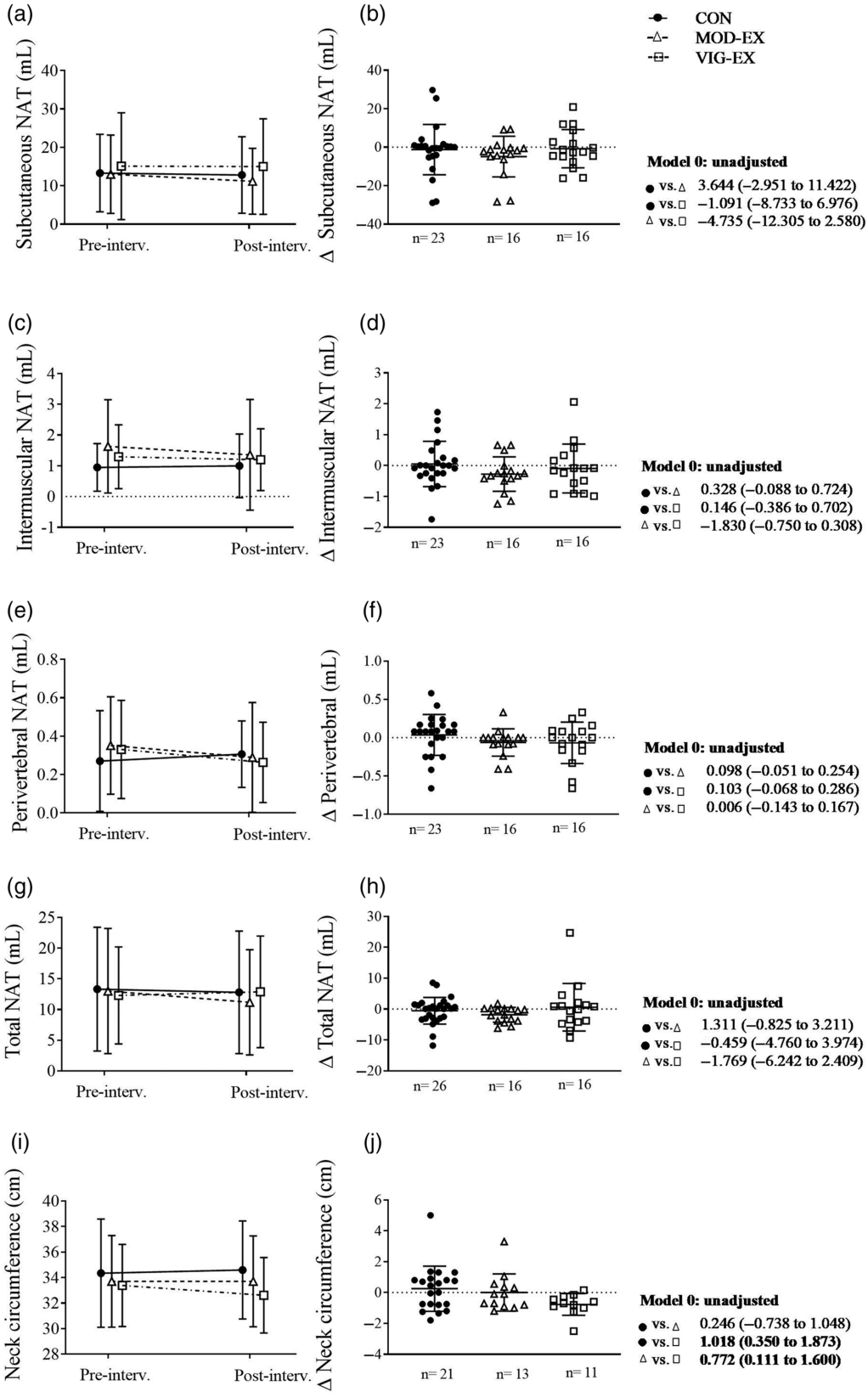

Figure 1 — Effect of the 24-week concurrent exercise intervention on the compartmental (subcutaneous, intermuscular, and perivertebral) and total NAT volumes and neck circumference. Only the exercisers who attended more than 70% of total training sessions and adhered to the training intensity established for each group (explained in statistical analysis section) were included. The presented model was not adjusted for any confounder (raw model, Model 0). The left panels (a, c, e, g, and i) show the mean value ± *SD* of the neck measurements before and after the intervention. The right panels (b, d, f, h, and j) show the mean and *SE* (as well as the scatterplot) of the change in neck measurements after the intervention (Postintervention – Preintervention values). Bootstrapping ANOVA was used to analyze the dose–response effect with no adjustments (Model 0). Bootstraps for pairwise comparisons were used to detect differences among groups. Mean differences and 95% bias-corrected-and-accelerated confidence intervals (based on 1,000 bootstrap samples) are provided. Significant differences ($p \leq .05$) are highlighted in bold. ANOVA = analysis of variance; CON = control group; MOD-EX = moderate-intensity exercise group; NAT = neck adipose tissue; VIG-EX = vigorous-intensity exercise group.

Table 2 Association of the Changes in Compartmental and Total NAT and Neck Circumference With Changes in Other Body Composition Variables, Cardiometabolic Risk, and the Inflammatory Profile in Young Adults

	Δ Subcutaneous NAT (ml)					Δ Intermuscular NAT (ml)					Δ Perivertebral NAT (ml)					Δ Total NAT (ml)					Δ Neck circumference (cm)				
	n	*B*	*SE*	Adj. R^2	*p*	*n*	*B*	*SE*	Adj. R^2	*p*	*n*	*B*	*SE*	Adj. R^2	*p*	*n*	*B*	*SE*	Adj. R^2	*p*	*n*	*B*	*SE*	Adj. R^2	*p*
Body composition																									
Δ Weight (kg)	70	**0.074**	0.034	.06	**.04**	70	0.676	0.638	.02	.29	70	0.800	3.103	0	.80	77	**0.217**	0.096	.11	**.03**	61	**1.371**	0.561	.09	**.02**
Δ Lean mass (kg)	70	6.261	19.957	.09	.75	70	110.0	467.4	.09	.81	70	895.3	1479.1	0.10	.54	77	67.129	59.344	.08	.26	61	−31.78	351.4	.04	.93
Δ Fat mass (kg)	70	48.062	38.530	.06	.22	70	481.6	556.8	.04	.39	70	−1231.4	2283.5	0.04	.59	77	**226.43**	90.421	.12	**.01**	61	**1196.0**	503.2	.15	**.02**
Δ Fat percentage (%)	70	0.469	0.034	.08	.18	70	0.151	0.400	.07	.71	70	0.726	1.806	0.07	.69	77	0.097	0.092	.08	.29	61	**1.130**	0.353	.21	**.002**
Δ VAT mass (g)	70	1.046	0.626	.08	.10	70	8.651	8.571	.07	.32	70	−34.02	41.220	0.07	.41	77	4.387	2.526	.05	.09	61	19.54	14.92	.09	.20
Cardiometabolic profile																									
Δ Glucose (mg/dl)	69	0.063	0.115	.05	.59	69	1.724	1.538	.05	.27	69	6.756	5.229	0.06	.20	75	0.340	0 .178	.07	.06	60	0.488	1.088	.01	.66
Δ Insulin (μIU/ml)	69	0.087	0 .058	.07	.14	69	−0.121	0.618	.04	.84	69	2.220	2.539	0.04	.38	75	0.161	0.101	.05	.11	60	0.165	0.414	.02	.69
Δ HOMA-IR	69	0.010	0.015	.06	.53	69	−0.027	0.141	.03	.85	69	0.473	0.651	0.04	.47	75	0.040	0.026	.04	.13	60	0 .057	0 .112	.01	.61
Δ TC (mg/dl)	69	**−0.660**	0 .321	.06	**.04**	69	−3.053	4.049	.02	.45	69	3.704	14.042	0.02	.79	75	−0.285	0.633	.00	.65	60	2.380	4.050	.03	.56
Δ LDL-C (mg/dl)	69	**−0.493**	0.250	.08	**.05**	69	−0.862	3.973	.05	.86	69	14.925	12.132	0.07	.22	75	−0.415	0.684	.01	.55	60	4.063	3.536	.06	.26
Δ HDL-C (mg/dl)	69	−0.099	0.138	.04	.47	69	−0.555	1.198	.03	.64	69	0	3.542	0.03	1.00	75	−0.182	0.152	.07	.23	60	−0.333	1.467	.04	.82
Δ TC/HDL-C	69	0.006	0.005	.05	.22	69	0.028	0.010	.04	0.78	69	0 .370	0 .296	0.07	.22	75	**0.023**	0.009	.11	**.01**	60	0.048	0 .070	.04	.49
Δ LDL-C/HDL-C	69	0.005	0.005	.07	.31	69	0.013	0.070	.05	.85	69	0 .316	0 .244	0.08	.20	75	0.016	0.011	.10	.16	60	0.044	0.061	.04	.48
Δ Triglycerides (mg/dl)	69	0	0 .418	.03	1.00	69	−2.242	6.672	.04	0.74	69	15.625	25.057	0.04	.53	75	0 .396	0 .732	.05	.59	60	2.782	3.430	.02	.42
Δ SBP (mmHg)	64	−0.082	0 .118	.01	.49	64	−0.255	1.752	.01	.88	64	3.000	6.731	0.01	.66	71	−0.219	0.267	.02	.41	53	1.504	1.556	.06	.34
Δ DBP (mmHg)	64	−0.129	0.101	.04	.21	64	**−2.530**	1.072	.05	**.02**	64	2.040	6.066	0.02	.74	71	−0.086	0.267	.01	.75	53	1.25	1.372	.03	.37
Δ Muscular strength (kg)*	61	−0.000	0.001	.06	.45	61	−0.007	0.008	.08	.36	61	−0.025	0.029	0.08	.38	66	−0.011	0.000	.099	.17	61	−0.006	0.064	.15	.36
Δ CRF (ml/min)*	67	−0.58	0.434	.09	.90	67	12.035	91.83	.09	.90	67	−75.563	275.08	0.09	.78	73	3.596	8.360	.139	.67	59	60.655	44.853	.16	.18
Δ CMR-score	63	0.001	0.027	.03	.96	63	−0.061	0.324	.03	.85	63	1.174	1.994	0.03	.56	68	0.037	0.042	.05	.37	52	**0.556**	0.245	.09	**.03**
Inflammatory profile																									
Δ C-reactive protein (mg/L)	69	0.009	0.018	.01	.61	69	0.162	0.086	.03	.07	69	0.408	0.600	0.02	.50	75	0 .408	0.596	.02	.50	60	0	0.209	.04	1.00
Δ IL-2 (pg/ml)	69	−.0029	0.018	.02	.87	69	−0.020	0.222	.01	.93	69	0 .534	0.927	0.02	.57	76	0.534	0.930	.02	.57	60	0.075	0 .211	.03	.72
Δ IL-4 (pg/ml)	69	−0.041	0.103	.04	.69	69	−0.530	1.552	.04	.73	69	−3.538	4.202	0.04	.40	76	−3.538	4.202	.04	.40	60	0.630	0 .697	.03	.37
Δ IL-6 (pg/ml)	69	−.0075	0.011	.03	.48	69	−0.039	0 .133	.03	.77	69	−0.018	0.550	0.09	.97	76	−0.018	0.546	.02	.97	60	0.145	0.120	.06	.22
Δ IL-7 (pg/ml)	69	−0.002	0 .028	.00	.94	69	−0.174	0.293	.01	.55	69	−1.055	0 .842	0.01	.21	76	−1.055	0.842	.01	.21	60	−0.113	0.138	.07	.41
Δ IL-8 (pg/ml)	69	−0.002	0.009	.01	.87	69	−0.045	0.122	.01	.71	69	−0.072	0.460	0.01	.87	76	−0.072	0 .459	.01	.87	60	−0.018	0.093	.01	.84
Δ IL-10 (pg/ml)	69	0	0 .028	.01	1.00	69	0	0.375	.01	1.00	69	0	1.410	0.01	1.00	76	0	1.410	.01	1.00	60	0.952	0.909	.03	.30
Δ IL-17a (pg/ml)	69	−0.020	0 .043	.02	.64	69	−0.109	0 .404	.01	.79	69	−2.322	1.794	0.04	.20	76	−2.322	1.794	.04	.20	60	0.304	0.473	.02	.52
Δ IFNγ (pg/ml)	69	0 .007	0.111	.02	.95	69	−0.379	1.031	.03	.71	69	−0.473	3.834	0.02	.90	76	−0.473	3.834	.02	.90	60	−0.124	1.080	.03	.91
Δ TNFα (pg/ml)	69	−0.001	0.016	.00	.97	69	−0.004	0.200	.00	.98	69	0.217	0 .747	0.00	.77	76	0.217	0.747	0.00	.77	60	0.066	0.178	.03	.71
Δ Complement 3 (mg/dl)	69	0.112	0.173	.02	.50	69	−0.037	2.709	.02	.99	69	4.328	8.789	0.02	.62	75	4.328	8.789	.02	.62	60	0 .038	1.739	.11	.98
Δ Complement 4 (mg/dl)	69	0 .024	0.068	.01	.73	69	1.435	0 .763	.04	.06	69	3.733	3.696	0.02	.32	75	3.733	3.696	.02	.32	60	−0.732	0 .780	.08	.35
Δ β-Microglobulin 2 (mg/L)	70	0 .002	0.002	.04	.37	70	0.027	0 .024	.04	.27	70	−0.111	0.092	0.04	.23	77	−0.111	0 .092	.04	.23	61	−0.038	0 .024	.06	.12
Δ Adiponectin (mg/L)	69	−0.047	0.067	.02	.48	69	−0.550	0.716	.02	.45	69	−4.818	3.352	0.05	.15	75	−4.818	3.351	.05	.15	59	0.755	0.476	.05	.20
Δ Leptin (μg/L)	69	0.037	0.029	.02	.22	69	0 .219	0.241	.01	.37	69	1.358	1.160	0.01	.24	74	**0.101**	0 .050	.05	**.05**	58	0 .216	0.327	.04	.51

Note. Interquartile regressions were performed to examine the association of the changes in the neck measurements with changes in other body composition variables, cardiometabolic risk, and the inflammatory profile, after adjusting for the intervention group and sex (also for the baseline lean mass when indicated by the symbol *). All participants who attended ≥70% of the total training sessions were included in these analyses. The nonstandardized β coefficient (*B*), *SE*, adjusted *R*-squared (R^2), and *p* value are provided. Significant associations ($p \leq .05$) are highlighted in bold. After adjusting for the multiple comparisons error (familywise error rate: Hochberg procedure), all associations became nonsignificant (all $p > .05$), except for the association between the change in neck circumference with the change in percentage fat ($p = .05$). CMR = cardiometabolic risk; CRF = cardiorespiratory fitness; DBP = diastolic blood pressure; HDL = high-density lipoprotein cholesterol; HOMA-IR = homeostatic model assessment of insulin resistance; IFNγ = interferon-γ; IL = interleukin; LDL-C = low-density lipoprotein cholesterol; NAT = neck adipose tissue; SBP = systolic blood pressure; TC = total cholesterol; TNFα = tumor necrosis factor α; VAT = visceral adipose tissue.

quantify their upper body adipose tissue (and had at least one valid NAT measure) before the intervention. Among these, 105 participants finished the 24-week trial (CON: $n = 33$, MOD-EX: $n = 36$, and VIG-EX: $n = 36$) and underwent positron emission tomography combined with computed tomography (PET-CT) scanning again to have their NAT volume and neck circumference quantified. After excluding those participants who did not meet the training attendance or exercise intensity criteria, or who did not have at least one valid neck measurement, a total of 74 participants were included in the main intervention analyses (CON: $n = 34$, MOD-EX: $n = 19$, and VIG-EX: $n = 21$). Of note, average training attendance was 87%.

Table 1 shows the descriptive characteristics of the study subjects before and after the intervention. Age and values for anthropometry and body composition variables (including NAT measurements) were similar across the groups at baseline (all $p > .05$), although there was a larger proportion of women than men in all groups.

The 24-Week Concurrent Exercise Training Program Did Not Reduce Total or Compartmental NAT Volumes, but Slightly Reduced Neck Circumference in the VIG-EX Group

The exercise program did not reduce the compartmental or total NAT volumes (Figure 1a–1h), however, it significantly reduced neck circumference (Figure 1i–1j) in the VIG-EX group compared with the CON (mean change: 1.018, 95% bias-corrected-and-accelerated confidence intervals, 0.350 to 1.873 cm) and MOD-EX groups (0.772, 0.111 to 1.600 cm), when no adjustments were made. These differences remained (data not shown) after adjusting the analyses for the baseline values of the respective outcome (Model 1) or the baseline values plus sex (Model 2). When these analyses were repeated using NAT% instead of total NAT, no effect of the exercise program was seen. Daily energy and macronutrient intake did not influence these results (data not shown).

Overall, the exercise intervention had no effect on neck lean volume ($p > .05$; see Figure S2 in the Supplementary Material [available online]). The members of the VIG-EX group experienced a greater reduction in VAT mass compared with the CON group (as previously observed for neck circumference; see Figure S3 in the Supplementary Material [available online]). However, the latter effects were not robust and disappeared after adjusting for sex plus baseline values (Model 2; see Figures S2 and S3 in the Supplementary Material [available online]).

Relationship Between Changes in NAT Volume and Neck Circumference and Changes in Body Weight and Composition, and Cardiometabolic and Inflammatory Profiles

The change in total NAT volume was positively associated with the change in body weight (nonstandardized β coefficient: $B = 0.217$, adjusted R^2: $R^2 = .11$, $p = .03$) and in fat mass, TC/HDL-C, and leptin ($B = 226.43$, $R^2 = .12$, $p = .01$; $B = 0.023$, $R^2 = .11$, $p = .01$; $B = 0.101$, $R^2 = .05$, $p = .05$, respectively; Table 2); independently of the exercise intervention and sex. Similarly, the change in neck circumference was positively related to changes in body weight and fat mass ($B = 1.371$, $R^2 = .09$, $p = .02$; $B = 1{,}196.0$, $R^2 = .15$, $p = .02$, respectively), but also in percentage fat and CMR score ($B = 1.130$, $R^2 = .21$, $p = .002$; $B = 0.556$, $R^2 = .09$, $p = .03$, respectively). When NAT analyses were additionally adjusted by VAT mass, results remain overall unaltered, except for the relationship of the changes in total NAT with those in fat mass and leptin, which became nonsignificant ($p > .21$). When the relationships between changes in NAT volume and neck circumference with body composition and CMR/inflammatory profile were adjusted for multiplicity, all associations (except for that of neck circumference change with percentage fat change) became nonsignificant ($p > .05$, see legend to Table 2). Changes in neck lean volume were positively associated with changes in total cholesterol, LDL-C, and diastolic blood pressure ($B = 0.273$, $R^2 = .042$, $p = .03$; $B = 0.251$, $R^2 = .072$, $p = .03$; $B = 0.105$, $R^2 = .055$, $p = .02$, respectively; see Table S1 in the Supplementary Material [available online]).

Changes in VAT mass were positively related to changes in body weight ($B = 0.023$, $R^2 = .16$, $p < .001$), fat mass ($B = 32.32$, $R^2 = .04$, $p < .001$), percentage fat ($B = 0.029$, $R^2 = .38$, $p < .001$), CMR score ($B = 0.009$, $R^2 = .10$, $p = .001$), and the leptin concentration ($B = 0.006$, $R^2 = .04$, $p = .006$).

The predictive value of baseline neck measurements with respect to changes in other body composition variables, CMR and the inflammatory profile, was examined after adjusting for gender and intervention group (see Tables S3 and S4 in the Supplementary Material [available online]). Baseline subcutaneous and total NAT volumes were inversely related to changes in leptin concentration ($p < .05$, Table S3 in the Supplementary Material [available online]). However, the baseline neck lean volume seemed not to have any predictive value (all relationships $p > .05$, Table S4 in the Supplementary Material [available online]).

Discussion

In contrast to what was expected, the 24-week concurrent exercise intervention did not reduce the compartmental nor total NAT volumes in the present young adults. However, it did slightly reduce neck circumference (0.8–1 cm) in the VIG-EX group. In addition, changes in total NAT and neck circumference were positively, albeit weakly, related to changes in body weight and adiposity (and with CMR in the case of neck circumference), independently of the exercise group and participants' sex. These associations were similar (although less robust) to those observed for VAT mass, but most of the former associations became nonsignificant when adjusted for the multiple comparison error. The present results therefore need to be interpreted with caution.

Exercise is a cornerstone in the treatment of many diseases due to its wide variety of benefits (Fiuza-Luces et al., 2013). Previous evidence has shown that exercise benefits are not only mediated by increases in muscle mass and strength, but also by a reduction of whole-body adiposity and lipid deposition in ectopic depots, such as the VAT or hepatic fat, the amounts of which are directly related to CMR, and the inflammatory profile (Hong et al., 2014; Houghton et al., 2017; Mendelson et al., 2015; O'Leary et al., 2006; Taniguchi et al., 2016). NAT is a nonsplanchnic upper body ectopic fat depot that might increase CMR and promote a proinflammatory status in healthy people as well as in those with different diseases (Arias-Tellez & Acosta, 2021; Torriani et al., 2014). Interestingly, previous *in vivo* studies have shown that upper body fat is a major contributor to available systemic free fatty acids (Nielsen et al., 2004), and *in vitro* studies have shown that upper body adipocytes isolated from lean and obese participants are more responsive to lipolytic adrenergic stimulation than are lower

body adipocytes (Engfeldt & Arner, 1988). Altogether, this suggests that upper body fat depots have a greater propensity to mobilize fatty acids under lipolytic stimulus, such as exercise. Accordingly, we recently showed moderate intensity and/or overall PA to be related to lower intermuscular and total NAT in young men (Arias-Tellez et al., 2021), but the cross-sectional design of that study precluded conclusions on causality being drawn. In the present study, it was hypothesized that exercise would reduce the compartmental and total NAT volumes in a dose-dependent fashion, and that changes would be related to a lower CMR and better inflammation status. However, exercise appeared to lack any effect on NAT and its distribution, a finding that remained after adjustment for confounders. It is noteworthy that when these analyses were repeated (a) using a stricter criterion regarding attendance to the training sessions (80% of all sessions instead of 70%) or (b) a less strict criterion in which only attendance to the training sessions (70% of all sessions) was considered without taking into account adherence to the training intensity (see Figures S4 and S5 in the Supplementary Material [available online], respectively), similar results were obtained.

The absence of any previous evidence regarding the influence of exercise on NAT accumulation precluded any comparison with other studies being made. The lack of effect seen in the present work might be explained in the following ways. (a) The members of the studied population, who were young and relatively healthy, are likely to show less NAT accumulation than older or more obese participants, hampering the detection of any small, exercise-induced changes in this population. This is supported by the fact that exercise reduced the VAT mass (a well-known exercise effect) only in the VIG-EX group, and even then only slightly and with a wide variation in the results obtained. (b) There appears to be wide variation in NAT accumulation across different depots (Arias-Tellez & Acosta, 2021), and the results are also likely subject to "technical" variations (i.e., with respect to the time or manner the analysis was performed, etc., see Appendix B in the Supplementary Material [available online]). These technical variations probably introduce noise that could be reduced by developing standardized and validated protocols for analyzing NAT accumulation and its distribution.

Exercise reduced neck circumference only in the VIG-EX group (by approximately 1 cm compared with the CON group). Neck circumference is strongly correlated to compartmental and total NAT (Arias-Tellez & Acosta, 2021) and would appear to be a practical and valid tool—especially in the clinical context—for measuring obesity, CMR, and (potentially) inflammation across different ages and populations (Arias-Tellez & Acosta, 2021; Arias-Tellez et al., 2021; Wan et al., 2020; Yang et al., 2021). The incorporation of neck circumference into clinical trials might also facilitate information on the above variable in an easy, effective, and inexpensive way. However, evidence in this topic is controversial, since several studies have shown that exercise reduces neck circumference, whereas other have shown no effect (Ackel-D'Elia et al., 2012; de Oliveira Silva et al., 2018; Desplan et al., 2014; Kline et al., 2011; Zanetti et al., 2016).

Most of the studies carried out in this area have been of short duration, have involved fixed intensity exercise interventions, have mainly targeted the endurance or resistance components of exercise (instead of both together), and have focused on unhealthy populations (i.e., participants with obesity, metabolic syndrome, obstructive sleep apnea, etc.). The present results provide novel evidence that a long-term concurrent exercise intervention can slightly reduce neck circumference in young adults who partake in vigorous-intensity exercise, which may be related, together with other changes in body composition, to health benefits. This is supported in that changes in neck circumference were here seen to be related to changes in whole-body weight and adiposity, along with a reduction in CMR. While the exercise intervention did not reduce NAT accumulation, changes in total NAT were related to changes in body weight and adiposity and leptin concentrations, although these associations were not consistent. Future studies should investigate whether NAT accumulation is involved in CMR and metabolic dysfunction.

The present results cannot be generalized to people with excess upper body fat given the difficulties of accurately outlining the ROIs for distinguishing specific NAT compartments. In fact, since a thin pillow was placed under the head, which was therefore slightly inclined, ROIs for estimating the NAT volumes could only be drawn for the posterior part of the neck around the level of C5. Neither did the small number of participants in each group allow for separate analyses based on sex, which prevented full account being taken of the potential effect of sex on the variables of interest. Future studies with different types of exercise intervention and target populations (especially with older or less healthy populations where exercise effects may be larger), larger samples sizes, and with NAT accumulation as a primary outcome (this was an exploratory study), are warranted. Such studies should aim to examine the molecular signature of NAT (e.g., gene expression related to lipid metabolism and regulation) in an attempt to understand by which mechanisms exercise affects NAT accumulation, and how this NAT accumulation might be related to CMR and inflammation.

In conclusion, the present 24-week concurrent exercise intervention did not reduce NAT in young, relatively healthy adults. However, it did slightly reduced neck circumference in the VIG-EX group. In addition, changes in total NAT volume and neck circumference were associated with changes in body weight and whole-body adiposity, as well as in CMR (for neck circumference), although these associations were not consistent.

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