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

YEAR 2023

VERSION Final Draft

JOURNAL International Journal of Sport Nutrition and Exercise Metabolism

Effect of a 24-week concurrent exercise intervention on neck adiposity and its distribution in young adults: the ACTIBATE randomized controlled trial.

Running Head: Exercise and neck adipose tissue

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ABSTRACT

Background: Neck adipose tissue (NAT) accumulation and neck circumference are independent predictors of cardiometabolic risk (CMR) and 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.

Methods: Seventy-four subjects (51 women, age 22±2 years) were included in the main analyses, after being randomly assigned to either a: i) control (n=34, CON), ii) moderate intensity exercise (n=19, MOD-EX), or iii) vigorous intensity exercise (n=21, VIG-EX) group. Subjects in the exercise groups trained 3-4 days/week (endurance + resistance exercise training). NAT volume and NAT distribution across different depots was 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.

Results: The exercise intervention did not reduce the total NAT volume, nor was NAT distribution affected ($P>0.05$). However, it did reduce neck circumference in the VIG-EX group compared to the MOD-EX and CON groups (by 0.8 and 1 cm, respectively, $P\leq0.05$). Changes in total NAT and neck circumference were positively, albeit weakly, related (adj. R^2: 0.05-0.21, all $P\leq0.05$) to changes in body weight and adiposity, leptin (only total NAT), and CMR (only neck circumference).

Conclusion: 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.

INTRODUCTION

The last decade has seen attention focused on previously overlooked adipose tissue depots that might influence cardiometabolic risk (CMR). This has shown that the accumulation of neck adipose tissue (NAT) - a heterogeneous and anatomically disperse depot within the non-splanchnic upper body fat - is related to CMR independent of body mass index (BMI) or visceral adipose tissue (VAT) mass in patients with successfully treated malignant/benign tumours (1-3). Indeed, it has also been associated with all-cause mortality (4). In addition, our group has reported that NAT accumulation and a larger neck circumference are related to poorer CMR and inflammatory profiles, independent of BMI or VAT mass, in young and relatively healthy adults (relationships stronger in men and dependent on NAT compartmental distribution) (5).

Physical activity appears to be one of the major factors affecting NAT accumulation and distribution. We recently showed that moderate intensity and/or overall physical activity are associated with lower intermuscular and total NAT in young men (6), 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 (7), 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 (8-10), and certainly the reduction of excessive visceral fat through exercise in people with obesity is related to reduced fasting plasma glucose, insulin, triglycerides, and insulin resistance values (10-14). There is, therefore, a need to determine whether being more physically active reduces NAT accumulation, and therefore contributes towards reducing CMR and improving the inflammatory profile.

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

RESEARCH DESIGN AND METHODS

Study Design

The present work is an ancillary study of the 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 (nº 924) and by that of the *Servicio Andaluz de Salud*. The study was performed in accordance with the Declaration of Helsinki (2013 revision). All subjects gave their written, informed consent to be included. Subject 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, subjects were enrolled in four different waves (16-24 subjects in each wave) starting in September-December.

Study subjects

The study protocol, the description of procedures and the inclusion-exclusion criteria are provided elsewhere (15). Briefly, all subjects 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 non-smokers, 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 subjects confirmed their interest in the project and were assessed for eligibility (see flowchart, **Figure S1**); 145 were finally enrolled.

Intervention

After baseline assessment of the primary and secondary outcome variables (see below), subjects were randomly assigned to either i) a control group (CON, no exercise, n=54), ii) a moderate intensity exercise group (MOD-EX, n=48), or a iii) vigorous intensity exercise group (VIG-EX, n=43). Subjects 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 elsewhere (15). 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 programme was based on the physical activity recommendations for adults proposed by the World Health Organization (16).

a) *Frequency*. Subjects came to the research centre 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. Hence, one or two sessions per week consisted solely of endurance exercise.

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% HRres (heart rate reserve), whereas the VIG-EX group performed 75 min/week at moderate intensity (i.e., 60% HRres) and 75 min/week at vigorous intensity (i.e., 80% HRres).
- 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.

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 pre-specified 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 (15). All subjects wore an RS800CX exercise heart rate monitor (Polar Electro Öy, Kempele, Finland) during the exercise sessions in order to monitor exercise intensity.

Training sessions were distributed over the day. The subjects had the option to choose a schedule that best suited them, and were asked to keep to it throughout the intervention period. Four schedules were possible: 8:30 to 10:30 am, 16:00 to 18:00 pm, 18:00 to 20:00 pm, and 20:00 to 22:00 pm. 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 centre), subjects were provided with a pulsometer, an elastic band and specific instructions to carry out adapted training sessions at home. Subjects 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 computed tomography scanning were performed using a Biograph 16 PET-CT scanner (Siemens, Erlangen, German) from the atlas vertebra to the mid-chest region. Briefly, all subjects confirmed that they had met the pre-requisites of: i) arriving in a fasting state (at least 6 h), ii) having slept as usual, iii) having refrained from any moderate or vigorous physical activity (within 24 and 48 h respectively), iv) not having consumed any alcoholic or stimulant beverages in the previous 6 h or taken any drugs in the last 24 h. The original aim of the ACTIBATE study (17) was to detect the ^{18}F-FDG uptake of brown adipose tissue (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 (18). After 1 h, 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 http://sourceforge.net/projects/bifijiplugins/. Using a 3D-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 (HU).

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, semiespinalis 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 HU and 9 to 150 HU were used to determine the total NAT and neck lean volumes respectively (the latter including skeletal muscle tissue, blood vessels, and some internal organs) (19). A detailed description of the analytical procedures is reported elsewhere (5). The percentage of total NAT within this ROI was also calculated (5).

Neck circumference was measured perpendicular to the longitudinal axis of the neck using an inextensible metallic tape running over the thyroid cartilage (20). The subjects 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 skilful anthropometrist) (21).

Secondary outcomes

Anthropometry and body composition

Weight and height were measured using a model 769 calibrated digital scale and a portable model 213 stadiometer respectively (both from SECA, Hamburg, Germany). BMI was then calculated as *body weight (kg)/height squared (m^2)*. Waist circumference was measured at the minimum perimeter; when subjects showed abdominal obesity it was determined in a horizontal plane above the umbilicus (20).

A Discovery Wi dual-energy X-ray absorptiometry (DXA) scanner (Hologic, Inc., Bedford, MA, USA) was used to determine total body lean mass, fat mass and VAT mass. The lean and fat mass and the percentage fat mass of the trunk and appendicular regions were determined (22).

Cardiometabolic and inflammatory profile

CMR and inflammation markers were normally assessed within three weeks of the ^{18}F-FDG-PET/CT assessment. Subjects came to our facilities in the morning for the extraction of blood samples, and were asked to confirm having met the requirements of i) arriving in a fasting state (10–14 h), ii) having slept as usual, iii) having refrained from any kind of physical

activity during the morning when the blood extractions were performed (all had to come by motorized transport), iv) having refrained from any moderate or vigorous physical activity (within 24 and 48 h respectively), and v) not having consumed any stimulant beverage.

Serum glycaemic (glucose and insulin) and lipid markers (total cholesterol, high-density lipoprotein-cholesterol [HDL-C], low-density lipoprotein-cholesterol [LDL-C], and triglycerides) were determined. The homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated as *insulin [µU/mL] x glucose [mmol/L]/22.5* (23). The CMR score was computed using the variables outlined elsewhere (24) (i.e., waist circumference, blood pressure, glucose, and HDL-C and triglyceride concentrations). Details regarding how these variables were determined and the ensuing calculations are shown in **Supplementary Material – Appendix A.**

Serum/plasma pro- and anti-inflammatory markers (in serum = C-reactive protein, C3, C4, β-microglobulin; in plasma = interleukin [IL]-2, IL-4, IL-6, IL-7, IL-8, IL-10, IL-17a, interferon gamma [IFNɣ], tumour necrosis factor-alpha [TNF-α], adiponectin and leptin concentrations) were also measured using standard methods or specific Luminex kits as detailed in **Supplementary Material – Appendix A.**

Energy and macronutrient intake

Energy and macronutrient intake were calculated from 24 h dietary recalls as reported elsewhere.

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.

Randomisation and blinding

Eligible subjects were randomly assigned to the control or exercise groups (after completing all baseline assessments) using a simple, unrestricted randomization program (25). The principal investigator (JRR) was the only person aware of the subjects' group assignments; this information was only communicated to the rest of the research team once an eligible subject was ready to start the intervention phase.

Statistical analysis

The characteristics of the study subjects were recorded using descriptive statistics **(Table 1**). Mean and standard deviation or median values and interquartile ranges (percentile 25 to 75) are provided for normally and non-normally distributed variables, respectively. Differences among the groups at baseline were examined by one-way analysis of variance (ANOVA) for normally distributed variables, and Kruskal-Wallis tests for non-normally distributed variables. The influence of the interaction *exercise group x subject gender* 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 programme on NAT volume and neck circumference, and considering that most variables were not normally distributed, bootstrapping ANOVA was used to analyse 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 (95% BCaCI, based on 1000 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% HRres) and <20% at transition intensity (65-75% HRes) for the MOD-EX group, and as >40% of the total training time at vigorous intensity (>75% HRes) 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**), and VAT mass (as a reference tissue, **Table S2**) 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.

Non-standardized 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-26.0 software (IBM, NY, USA), except for the interquartile

regressions, which were performed using STATA software (StataCorp, Texas, USA). Significance was set at $P \leq 0.05$.

RESULTS

As shown in **Figure S1** (flowchart), of the 194 subjects 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 subjects underwent the ^{18}F-FDG-PET-CT scan to quantify their upper body adipose tissue (and had at least one valid NAT measure) before the intervention. Among these, 105 subjects finished the 24-week trial (CON: n=33, MOD-EX: n=36, VIG-EX: n=36) and underwent PET-CT scanning again to have their NAT volume and neck circumference quantified. After excluding those subjects 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 subjects were included in the main intervention analyses (CON: n=34, MOD-EX: n=19, VIG-EX: n=21). Of note, average training attendance was X %.

Those subjects with no valid neck measurements (due to image analysis problems) were excluded from the analysis of the compartmental NAT. The most common problem occurred with subjects with excess upper body fat, for whom ROIs for distinguishing specific compartments could not be accurately drawn. Their CT scan resolutions were also poor. Other subjects were excluded from the total NAT analyses because the jowl was included in the ROI - a problem due to the subject's position during the PET/CT scan; it was impossible to exclude it from the outlined ROI.

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 $P>0.05$), although there was a larger proportion of women than men in all groups.

The 24-week concurrent exercise training programme 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 1, Panels A-H**). However, it significantly reduced neck circumference (**Figure 1, Panels I and J)** in the VIG-EX group compared to the CON (mean change: 1.018, 95% BCaCI 0.350 to 1.873 cm) and MOD-EX groups (0.772, 0.111 to 1600 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 gender (model 2). When these analyses were repeated using NAT% instead of total NAT, no effect of the exercise program was seen (either for the non-adjusted or adjusted models). Daily energy and macronutrient intake neither seemed to influence these results (data not shown).

Overall, the exercise intervention had no effect on neck lean volume (P>0.05) (**Figure S2**), although the CON group showed a slight increase on this variable compared to the VIG-EX group. The members of the VIG-EX group experienced a greater reduction in VAT mass compared to the CON group (as previously observed for neck circumference) **(Figure S3**). However, the latter effects were not robust and disappeared after adjusting for gender plus baseline values (model 2) (**Figures S2 and S3**).

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 (non-standardized β coefficient: B=0.217, adjusted R^2: R^2=0.11, P-value: P=0.03) and in fat

mass, TC/HDL-C and leptin (B=226.43, R^2=0.12, P=0.01; B=0.023, R^2=0.11, P=0.01; B=0.101, R^2=0.05, P=0.05, respectively, **Table 2**); independently of the exercise intervention and gender. Similarly, the change in neck circumference was positively related to changes in body weight and fat mass (B=1.371, R^2=0.09, P=0.02; B=1196.0, R^2=0.15, P=0.02, respectively), but also in percentage fat and CMR score (B=1.130, R^2=0.21, P=0.002; B=0.556, R^2=0.09, P=0.03, 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 non-significant (P>0.21). 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 non-significant (P>0.05, **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=0.042, P=0.03; B=0.251, R^2=0.072, P=0.03; B=0.105, R^2=0.055, P=0.02, respectively) (**Table S1**).

The relationships between the change in VAT mass, and changes in body composition, CMR and the inflammatory profile, were also examined, adjusting for intervention group and gender (**Table S2**). Patterns similar to those observed for the total NAT and/or neck circumference were found. Thus, changes in VAT mass were positively related to changes in body weight (B=0.023, R^2=0.16, P<0.001), fat mass (B= 32.32, R^2=0.04, P<0.001), percentage fat (B=0.029, R^2=0.38, P<0.001), CMR score (B=0.009, R^2=0.10, P=0.001) and the leptin concentration (B=0.006, R^2=0.04, P=0.006).

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**). Baseline subcutaneous and total NAT volumes were inversely related to changes in leptin concentration ($P<0.05$, **Table S3**). However, the baseline neck lean volume seemed not to have any predictive value (all relationships $P>0.05$, **Table S4**).

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 non-significant 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 (7). 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 (10, 13, 14, 26, 27). NAT is a non-splanchnic upper body ectopic fat depot that might increase CMR and promote a pro-inflammatory status in healthy people as well as in those with different diseases (2, 5). Interestingly, previous *in*

vivo studies have shown that upper body fat is a major contributor to available systemic free fatty acids (28), and *in vitro* studies have shown that upper body adipocytes isolated from lean and obese subjects are more responsive to lipolytic adrenergic stimulation than are lower body adipocytes (29). Altogether, this suggests that upper body fat depots have a greater propensity to mobilize fatty acids under lipolytic stimulus, such as physical activity or exercise. Accordingly, we recently showed moderate intensity and/or overall PA to be related to lower intermuscular and total NAT in young men (6), 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 1) using a stricter criterion regarding attendance to the training sessions (80% of all sessions instead of 70%), or 2) 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**, 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. i) The members of the studied population, who were young and relatively healthy, are likely to show less NAT accumulation than older or more obese subjects, 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. ii) There appears to be wide variation in NAT accumulation across different depots (5), 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).** These technical variations probably introduce noise that could be reduced by developing standardized and validated protocols for analyzing NAT accumulation and its distribution. Currently, there is no consensus on how to analyze NAT variables. Future methodological studies should focus on optimizing and standardizing protocols that are valid, reliable, replicable and generalizable to different populations, thus allowing comparison between studies.

Exercise reduced neck circumference only in the VIG-EX group (by approximately 1 cm compared to the CON group). Neck circumference is strongly correlated to compartmental and total NAT (5), 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 (5, 6, 30, 31). 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 different exercise modalities (resistance or aerobic exercise) or multicomponent interventions reduce neck circumference, whereas other have shown no effect (32-36).

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., subjects with obesity, metabolic syndrome, OSA, 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 (they disappeared after correcting for the multiple comparison error). 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 subjects in each group allow for separate analyses based on gender, 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, 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.

Acknowledgment, Authorships, Declarations.

Authorships. Designed the study - MJAT, FAM, BMT, GSD, JMLL, JRR; conducted the research - MJAT, FAM, BMT, GSD, EMR; provided essential reagents or materials - JMLL, JRR; analysed data or performed statistical analysis - MJAT, FAM; wrote the manuscript - MJAT, FAM; critical review of the manuscript and scientific assistance - MJAT, FAM, BMT, EMR, GSD, JRR; had primary responsibility for the final content - JRR. All authors approved the final version of the paper.

Declarations of funding sources. This study was supported by the Spanish Ministry of Economy and Competitiveness via the *Fondo de Investigación Sanitaria del Instituto de Salud Carlos III* (PI13/01393) and PTA 12264-I, *Retos de la Sociedad* (DEP2016-79512-R) and the European Regional Development Fund (ERDF), the Spanish Ministry of Education (FPU 13/03410), the *Fundación Iberoamericana de Nutrición* (FINUT), the *Redes Temáticas de Investigación Cooperativa* RETIC (Red SAMID RD16/0022), the AstraZeneca HealthCare Foundation, the University of Granada *Plan Propio de Investigación 2016* (Excellence actions: Unit of Excellence on Exercise and Health [UCEES]) - and *Plan Propio de Investigación 2018* (*Programa Contratos-Puente, Junta de Andalucía, Consejería de Conocimiento, Investigación y Universidades* (ERDF, SOMM17/6107/UGR), the *Fundación*

Carolina (C.2016-574961), and the *Fundación Alfonso Martín Escudero*. This study is part of a PhD thesis presented within the framework of the Biomedicine Doctoral Studies Programme at the University of Granada, Spain.

Conflicts of interest. None to declare.

References

1. Pandzic Jaksic V, Grizelj D, Livun A, Boscic D, Ajduk M, Kusec R, et al. Neck adipose tissue - tying ties in metabolic disorders. Horm Mol Biol Clin Investig. 2018;33(2).
2. Torriani M, Gill, C. M., Daley, S., et al. Compartmental neck fat accumulation and its relation to cardiovascular risk and metabolic syndrome. The American journal of clinical nutrition. 2014;100(5):1244-51.
3. Rosenquist K, Therkelsen K, Massaro J, Hoffmann U, Fox C. Development and reproducibility of a computed tomography-based measurement for upper body subcutaneous neck fat. Journal of the American Heart Association. 2014;3(6):e000979.
4. Tal S, Litovchik I. The association between neck adiposity and long-term outcome. 2019;14(4):e0215538.
5. Arias-Tellez MJ, Acosta FM. Neck adipose tissue accumulation is associated with higher overall and central adiposity, a higher cardiometabolic risk, and a pro-inflammatory profile in young adults. 2021;45(4):733-45.
6. Arias-Tellez MJ, Acosta FM, Migueles JH, Pascual-Gamarra JM, Merchan-Ramirez E, de Lucena Martins CM, et al. Higher Physical Activity Is Related to Lower Neck Adiposity in Young Men, but to Higher Neck Adiposity in Young Women: An Exploratory Study. International journal of sport nutrition and exercise metabolism. 2021:1-9.
7. Fiuza-Luces C, Garatachea N, Berger NA, Lucia A. Exercise is the real polypill. Physiology (Bethesda, Md). 2013;28(5):330-58.
8. Zhang H, Tong TK, Qiu W, Zhang X, Zhou S, Liu Y. Comparable Effects of High-Intensity Interval Training and Prolonged Continuous Exercise Training on Abdominal Visceral Fat Reduction in Obese Young Women. 2017;2017:5071740.
9. Maillard F, Pereira B, Boisseau N. Effect of High-Intensity Interval Training on Total, Abdominal and Visceral Fat Mass: A Meta-Analysis. Sports medicine (Auckland, NZ). 2018;48(2):269-88.
10. Hong HR, Jeong JO, Kong JY, Lee SH, Yang SH, Ha CD, et al. Effect of walking exercise on abdominal fat, insulin resistance and serum cytokines in obese women. Journal of exercise nutrition & biochemistry. 2014;18(3):277-85.
11. Park HS, Lee K. Greater beneficial effects of visceral fat reduction compared with subcutaneous fat reduction on parameters of the metabolic syndrome: a study of weight reduction programmes in subjects with visceral and subcutaneous obesity. Diabetic medicine : a journal of the British Diabetic Association. 2005;22(3):266-72.
12. Ross R, Janssen I, Dawson J, Kungl AM, Kuk JL, Wong SL, et al. Exercise-induced reduction in obesity and insulin resistance in women: a randomized controlled trial. Obesity research. 2004;12(5):789-98.
13. O'Leary VB, Marchetti CM, Krishnan RK, Stetzer BP, Gonzalez F, Kirwan JP. Exercise-induced reversal of insulin resistance in obese elderly is associated with reduced visceral fat. Journal of applied physiology (Bethesda, Md : 1985). 2006;100(5):1584-9.
14. Mendelson M, Michallet AS, Monneret D, Perrin C, Estève F, Lombard PR, et al. Impact of exercise training without caloric restriction on inflammation, insulin resistance and visceral fat mass in obese adolescents. Pediatric obesity. 2015;10(4):311-9.
15. Sanchez-Delgado G, Martinez-Tellez B, Olza J, Aguilera CM, Labayen I, Ortega FB, et al. Activating brown adipose tissue through exercise (ACTIBATE) in young adults: Rationale, design and methodology. Contemporary clinical trials. 2015;45(Pt B):416-25.
16. Who W. Global recommendations on physical activity for health. Geneva: World Health Organization. 2010.

17. Sanchez-Delgado G, Martinez-Tellez, B., Olza, J., et al. Activating brown adipose tissue through exercise (ACTIBATE) in young adults: Rationale, design and methodology. Contemporary clinical trials. 2015;45(Pt B):416-25.
18. Martinez-Tellez B, Sanchez-Delgado G, Garcia-Rivero Y, Alcantara JMA, Martinez-Avila WD, Munoz-Hernandez MV, et al. A New Personalized Cooling Protocol to Activate Brown Adipose Tissue in Young Adults. Frontiers in physiology. 2017;8:863.
19. Chung H, Cobzas D, Birdsell L, Lieffers J, Baracos V, editors. Automated segmentation of muscle and adipose tissue on CT images for human body composition analysis. Medical Imaging 2009: Visualization, Image-Guided Procedures, and Modeling; 2009: International Society for Optics and Photonics.
20. Stewart A, Marfell-Jones M, Olds T, Ridder dH. International Society for Advancement of Kinanthropometry. International standards for anthropometric assessment Lower Hutt, New Zealand: International Society for the Advancement of Kinanthropometry. 2011:50-3.
21. Norton K, Olds T. Antropometrica. Argentina: Biosystem; 2000.
22. Carbone S, Billingsley HE, Rodriguez-Miguelez P, Kirkman DL, Garten R, Franco RL, et al. Lean Mass Abnormalities in Heart Failure: The Role of Sarcopenia, Sarcopenic Obesity, and Cachexia. Current problems in cardiology. 2019:100417.
23. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-9.
24. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation. 2009;120(16):1640-5.
25. Schulz KF, Grimes DA. Generation of allocation sequences in randomised trials: chance, not choice. Lancet (London, England). 2002;359(9305):515-9.
26. Houghton D, Thoma C, Hallsworth K, Cassidy S, Hardy T, Burt AD, et al. Exercise Reduces Liver Lipids and Visceral Adiposity in Patients With Nonalcoholic Steatohepatitis in a Randomized Controlled Trial. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2017;15(1):96-102.e3.
27. Taniguchi H, Tanisawa K, Sun X, Kubo T, Higuchi M. Endurance Exercise Reduces Hepatic Fat Content and Serum Fibroblast Growth Factor 21 Levels in Elderly Men. The Journal of clinical endocrinology and metabolism. 2016;101(1):191-8.
28. Nielsen S, Guo Z, Johnson CM, Hensrud DD, Jensen MD. Splanchnic lipolysis in human obesity. The Journal of clinical investigation. 2004;113(11):1582-8.
29. Engfeldt P, Arner P. Lipolysis in human adipocytes, effects of cell size, age and of regional differences. Hormone and metabolic research Supplement series. 1988;19:26-9.
30. Wan H, Wang Y, Xiang Q, Fang S, Chen Y, Chen C, et al. Associations between abdominal obesity indices and diabetic complications: Chinese visceral adiposity index and neck circumference. 2020;19(1):118.
31. Yang GR, Yuan MX, Wan G, Zhang XL, Fu HJ, Yuan SY, et al. Neck circumference and waist circumference associated with cardiovascular events in type 2 diabetes (Beijing Community Diabetes Study 23). Scientific reports. 2021;11(1):9491.
32. Zanetti HR, Cruz LG, Lourenco CL, Neves Fde F, Silva-Vergara ML, Mendes EL. Non-linear resistance training reduces inflammatory biomarkers in persons living with HIV: A randomized controlled trial. European journal of sport science. 2016;16(8):1232-9.

33. de Oliveira Silva A, Dutra MT, de Moraes W, Funghetto SS, Lopes de Farias D, Dos Santos PHF, et al. Resistance training-induced gains in muscle strength, body composition, and functional capacity are attenuated in elderly women with sarcopenic obesity. Clinical interventions in aging. 2018;13:411-7.
34. Desplan M, Mercier J, Sabate M, Ninot G, Prefaut C, Dauvilliers Y. A comprehensive rehabilitation program improves disease severity in patients with obstructive sleep apnea syndrome: a pilot randomized controlled study. Sleep medicine. 2014;15(8):906-12.
35. Ackel-D'Elia C, da Silva AC, Silva RS, Truksinas E, Sousa BS, Tufik S, et al. Effects of exercise training associated with continuous positive airway pressure treatment in patients with obstructive sleep apnea syndrome. Sleep & breathing = Schlaf & Atmung. 2012;16(3):723-35.
36. Kline CE, Crowley EP, Ewing GB, Burch JB, Blair SN, Durstine JL, et al. The effect of exercise training on obstructive sleep apnea and sleep quality: a randomized controlled trial. Sleep. 2011;34(12):1631-40.

Table 1. Descriptive characteristics of the study subjects.

	PRE-INTERVENTION								POST-INTERVENTION								
	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*	0.87
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*	0.94
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*	0.50
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*	0.98
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*	0.88
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*	0.44
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*	0.30
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*	0.66
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*	0.74
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*	0.28
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*	0.46
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*	0.97
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*	0.61
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*	0.83

Continuous variables are presented as means (standard deviation) 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 analysis of variance (ANOVA) for normally distributed variables, and the Kruskal-Wallis test for non-normally 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. CON: control group, NAT: neck adipose tissue, MOD- EX: moderate intensity exercise group, VAT: visceral 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-value	n	B	SE	Adj. R^2	P-value	n	B	SE	Adj. R^2	P-value	n	B	SE	Adj. R^2	P-value	n	B	SE	Adj. R^2	P-value
Body composition																									
Δ Weight (kg)	70	**0.074**	0.034	0.06	**0.04**	70	0.676	0.638	0 .02	0.29	70	0.800	3.103	0	0.80	77	**0.217**	0.096	0.11	**0.03**	61	**1.371**	0.561	0.09	**0.02**
Δ Lean mass (kg)	70	6.261	19.957	0.09	0.75	70	110.0	467.4	0.09	0.81	70	895.3	1479.1	0.10	0.54	77	67.129	59.344	0.08	0.26	61	-31.78	351.4	0.04	0.93
Δ Fat mass (kg)	70	48.062	38.530	0.06	0.22	70	481.6	556.8	0.04	0.39	70	-1231.4	2283.5	0.04	0.59	77	**226.43**	90.421	0.12	**0.01**	61	**1196.0**	503.2	0.15	**0.02**
Δ Fat percentage (%)	70	0.469	0.034	0.08	0.18	70	0.151	0.400	0.07	0.71	70	0.726	1.806	0.07	0.69	77	0.097	0.092	0.08	0.29	61	**1.130**	0.353	0.21	**0.002**
Δ VAT mass (g)	70	1.046	0.626	0.08	0.10	70	8.651	8.571	0.07	0.32	70	-34.02	41.220	0.07	0.41	77	4.387	2.526	0.05	0.09	61	19.54	14.92	0.09	0.20
Cardiometabolic profile																									
Δ Glucose (mg/dL)	69	0.063	0.115	0.05	0.59	69	1.724	1.538	0.05	0.27	69	6.756	5.229	0.06	0.20	75	0.340	0 .178	0.07	0.06	60	0.488	1.088	0.01	0.66
Δ Insulin (μIU/mL)	69	0.087	0 .058	0.07	0.14	69	-0.121	0.618	0.04	0.84	69	2.220	2.539	0.04	0.38	75	0.161	0.101	0.05	0.11	60	0.165	0.414	0.02	0.69
Δ HOMA-IR	69	0.010	0.015	0.06	0.53	69	-0.027	0.141	0.03	0.85	69	0.473	0.651	0.04	0.47	75	0.040	0.026	0.04	0.13	60	0 .057	0 .112	0.01	0.61
Δ TC (mg/dL)	69	**-0.660**	0 .321	0.06	**0.04**	69	-3.053	4.049	0.02	0.45	69	3.704	14.042	0.02	0.79	75	-0.285	0.633	0.00	0.65	60	2.380	4.050	0.03	0.56
Δ LDL-C (mg/dL)	69	**-0.493**	0.250	0.08	**0.05**	69	-0.862	3.973	0.05	0.86	69	14.925	12.132	0.07	0.22	75	-0.415	0.684	0.01	0.55	60	4.063	3.536	0.06	0.26
Δ HDL-C (mg/dL)	69	-0.099	0.138	0.04	0.47	69	-0.555	1.198	0.03	0.64	69	0	3.542	0.03	1.00	75	-0.182	0.152	0.07	0.23	60	-0.333	1.467	0.04	0.82
Δ TC/HDL-C	69	0.006	0.005	0.05	0.22	69	0.028	0.010	0.04	0.78	69	0 .370	0 .296	0.07	0.22	75	**0.023**	0.009	0.11	**0.01**	60	0.048	0 .070	0.04	0.49
Δ LDL-C/HDL-C	69	0.005	0.005	0.07	0.31	69	0.013	0.070	0.05	0.85	69	0 .316	0 .244	0.08	0.20	75	0.016	0.011	0.10	0.16	60	0.044	0.061	0.04	0.48
Δ Triglycerides (mg/dL)	69	0	0 .418	0.03	1.00	69	-2.242	6.672	0.04	0.74	69	15.625	25.057	0.04	0.53	75	0 .396	0 .732	0.05	0.59	60	2.782	3.430	0.02	0.42
Δ SBP (mmHg)	64	-0.082	0 .118	0.01	0.49	64	-0.255	1.752	0.01	0.88	64	3.000	6.731	0.01	0.66	71	-0.219	0.267	0.02	0.41	53	1.504	1.556	0.06	0.34
Δ DBP (mmHg)	64	-0.129	0.101	0.04	0.21	64	**-2.530**	1.072	0.05	**0.02**	64	2.040	6.066	0.02	0.74	71	-0.086	0.267	0.01	0.75	53	1.25	1.372	0.03	0.37
Δ Muscular strength (kg)*	61	-0.000	0.001	0.06	0.45	61	-0.007	0.008	0.08	0.36	61	-0.025	0.029	0.08	0.38	66	-0.011	0.000	0.099	0.17	61	-0.006	0.064	0.15	0.36
Δ CRF (ml/min)*	67	-0.58	0.434	0.09	0.90	67	12.035	91.83	0.09	0.90	67	-75.563	275.08	0.09	0.78	73	3.596	8.360	0.139	0.67	59	60.655	44.853	0.16	0.18
Δ CMR-score	63	0.001	0.027	0.03	0.96	63	-0.061	0.324	0.03	0.85	63	1.174	1.994	0.03	0.56	68	0.037	0.042	0.05	0.37	52	**0.556**	0.245	0.09	**0.03**
Inflammatory profile																									
Δ C-reactive protein (mg/L)	69	0.009	0.018	0.01	0.61	69	0.162	0.086	0.03	0.07	69	0.408	0.600	0.02	0.50	75	0 .408	0.596	0.02	0.50	60	0	0.209	0.04	1.00
Δ IL-2 (pg/mL)	69	-.0029	0.018	0.02	0.87	69	-0.020	0.222	0.01	0.93	69	0 .534	0.927	0.02	0.57	76	0.534	0.930	0.02	0.57	60	0.075	0 .211	0.03	0.72
Δ IL-4 (pg/mL)	69	-0.041	0.103	0.04	0.69	69	-0.530	1.552	0.04	0.73	69	-3.538	4.202	0.04	0.40	76	-3.538	4.202	0.04	0.40	60	0.630	0 .697	0.03	0.37
Δ IL-6 (pg/mL)	69	-.0075	0.011	0.03	0.48	69	-0.039	0 .133	0.03	0.77	69	-0.018	0.550	0.09	0.97	76	-0.018	0.546	0.02	0.97	60	0.145	0.120	0.06	0.22
Δ IL-7 (pg/mL)	69	-0.002	0 .028	0.00	0.94	69	-0.174	0.293	0.01	0.55	69	-1.055	0 .842	0.01	0.21	76	-1.055	0.842	0.01	0.21	60	-0.113	0.138	0.07	0.41
Δ IL-8 (pg/mL)	69	-0.002	0.009	0.01	0.87	69	-0.045	0.122	0.01	0.71	69	-0.072	0.460	0.01	0.87	76	-0.072	0 .459	0.01	0.87	60	-0.018	0.093	0.01	0.84
Δ IL-10 (pg/mL)	69	0	0 .028	0.01	1.00	69	0	0.375	0.01	1.00	69	0	1.410	0.01	1.00	76	0	1.410	0.01	1.00	60	0.952	0.909	0.03	0.30
Δ IL-17a (pg/mL)	69	-0.020	0 .043	0.02	0.64	69	-0.109	0 .404	0.01	0.79	69	-2.322	1.794	0.04	0.20	76	-2.322	1.794	0.04	0.20	60	0.304	0.473	0.02	0.52
Δ IFNɣ (pg/mL)	69	0 .007	0.111	0.02	0.95	69	-0.379	1.031	0.03	0.71	69	-0.473	3.834	0.02	0.90	76	-0.473	3.834	0.02	0.90	60	-0.124	1.080	0.03	0.91
Δ TNFα (pg/mL)	69	-0.001	0.016	0.00	0.97	69	-0.004	0.200	0.00	0.98	69	0.217	0 .747	0.00	0.77	76	0.217	0.747	0.00	0.77	60	0.066	0.178	0.03	0.71
Δ Complement 3 (mg/dL)	69	0.112	0.173	0.02	0.50	69	-0.037	2.709	0.02	0.99	69	4.328	8.789	0.02	0.62	75	4.328	8.789	0.02	0.62	60	0 .038	1.739	0.11	0.98
Δ Complement 4 (mg/dL)	69	0 .024	0.068	0.01	0.73	69	1.435	0 .763	0.04	0.06	69	3.733	3.696	0.02	0.32	75	3.733	3.696	0.02	0.32	60	-0.732	0 .780	0.08	0.35
Δ β-microglobulin 2 (mg/L)	70	0 .002	0.002	0.04	0.37	70	0.027	0 .024	0.04	0.27	70	-0.111	0.092	0.04	0.23	77	-0.111	0 .092	0.04	0.23	61	-0.038	0 .024	0.06	0.12
Δ Adiponectin (mg/L)	69	-0.047	0.067	0.02	0.48	69	-0.550	0.716	0.02	0.45	69	-4.818	3.352	0.05	0.15	75	-4.818	3.351	0.05	0.15	59	0.755	0.476	0.05	0.20
Δ Leptin (μg/L)	69	0.037	0.029	0.02	0.22	69	0 .219	0.241	0.01	0.37	69	1.358	1.160	0.01	0.24	74	**0.101**	0 .050	0.05	**0.05**	58	0 .216	0.327	0.04	0.51

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 subjects who attended ≥70% of the total training sessions were included in these analyses. The non-standardized β coefficient (B), standard deviation (SE), adjusted R squared (R^2), and P-value are provided. After adjusting for the multiple comparisons error (familywise error rate: Hochberg procedure), all associations became non-significant (all P>0.05), except for the association between the change in neck circumference with the change in percentage fat (P=0.05). CMR: cardiometabolic risk, CRF: cardiorespiratory fitness, CRF_{LM}: cardiorespiratory fitness relative to lean body mass, DBP: diastolic blood pressure, HDL: high-density lipoprotein-cholesterol, HOMA-IR: homeostatic model assessment of insulin resistance, IFNɣ: interferon-ɣ, IL: interleukin, LDL-C: low-density lipoprotein-cholesterol, NAT: neck adipose tissue, SBP: systolic blood pressure, TC: total cholesterol, TNFα: tumour-necrosis factor α, VAT: visceral adipose tissue.

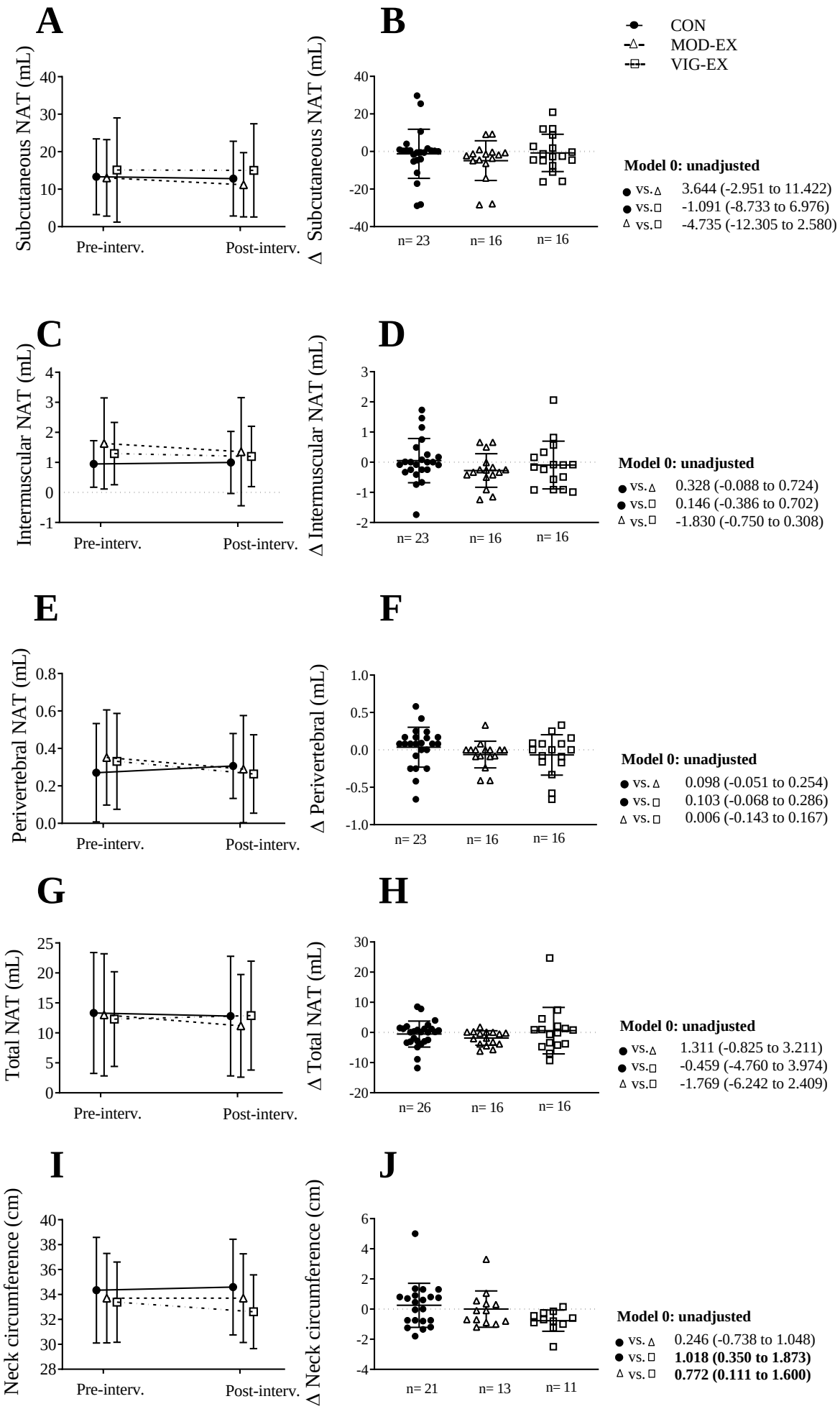
A
Subcutaneous NAT (mL)
40
30
20
10
0
Pre-interv.
Post-interv.
B
Δ Subcutaneous NAT (mL)
40
20
0
-20
-40
n= 23
n= 16
n= 16
CON
MOD-EX
VIG-EX
Model 0: unadjusted
● vs.△ 3.644 (-2.951 to 11.422)
● vs.□ -1.091 (-8.733 to 6.976)
△ vs.□ -4.735 (-12.305 to 2.580)
C
Intermuscular NAT (mL)
4
3
2
1
0
-1
Pre-interv.
Post-interv.
D
Δ Intermuscular NAT (mL)
3
2
1
0
-1
-2
n= 23
n= 16
n= 16
Model 0: unadjusted
● vs.△ 0.328 (-0.088 to 0.724)
● vs.□ 0.146 (-0.386 to 0.702)
△ vs.□ -1.830 (-0.750 to 0.308)
E
Perivertebral NAT (mL)
0.8
0.6
0.4
0.2
0.0
Pre-interv.
Post-interv.
F
Δ Perivertebral (mL)
1.0
0.5
0.0
-0.5
-1.0
n= 23
n= 16
n= 16
Model 0: unadjusted
● vs.△ 0.098 (-0.051 to 0.254)
● vs.□ 0.103 (-0.068 to 0.286)
△ vs.□ 0.006 (-0.143 to 0.167)
G
Total NAT (mL)
25
20
15
10
5
0
Pre-interv.
Post-interv.
H
Δ Total NAT (mL)
30
20
10
0
-10
-20
n= 26
n= 16
n= 16
Model 0: unadjusted
● vs.△ 1.311 (-0.825 to 3.211)
● vs.□ -0.459 (-4.760 to 3.974)
△ vs.□ -1.769 (-6.242 to 2.409)
I
Neck circumference (cm)
40
38
36
34
32
30
28
Pre-interv.
Post-interv.
J
Δ Neck circumference (cm)
6
4
2
0
-2
-4
n= 21
n= 13
n= 11
Model 0: unadjusted
● vs.△ 0.246 (-0.738 to 1.048)
● vs.□ 1.018 (0.350 to 1.873)
△ vs.□ 0.772 (0.111 to 1.600)

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 ± standard deviation of the neck measurements before and after the intervention. The right panels **(B, D, F, H and J)** show the mean and standard error (as well as the scatterplot) of the change in neck measurements after the intervention (POST intervention – PRE intervention values). Bootstrapping ANOVA was used to analyse 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 1000 bootstrap samples) are provided. Significant differences ($P \leq 0.05$) are highlighted in bold. CON: control group, MOD-EX: moderate intensity exercise group, NAT: neck adipose tissue, VIG-EX: vigorous intensity exercise group.