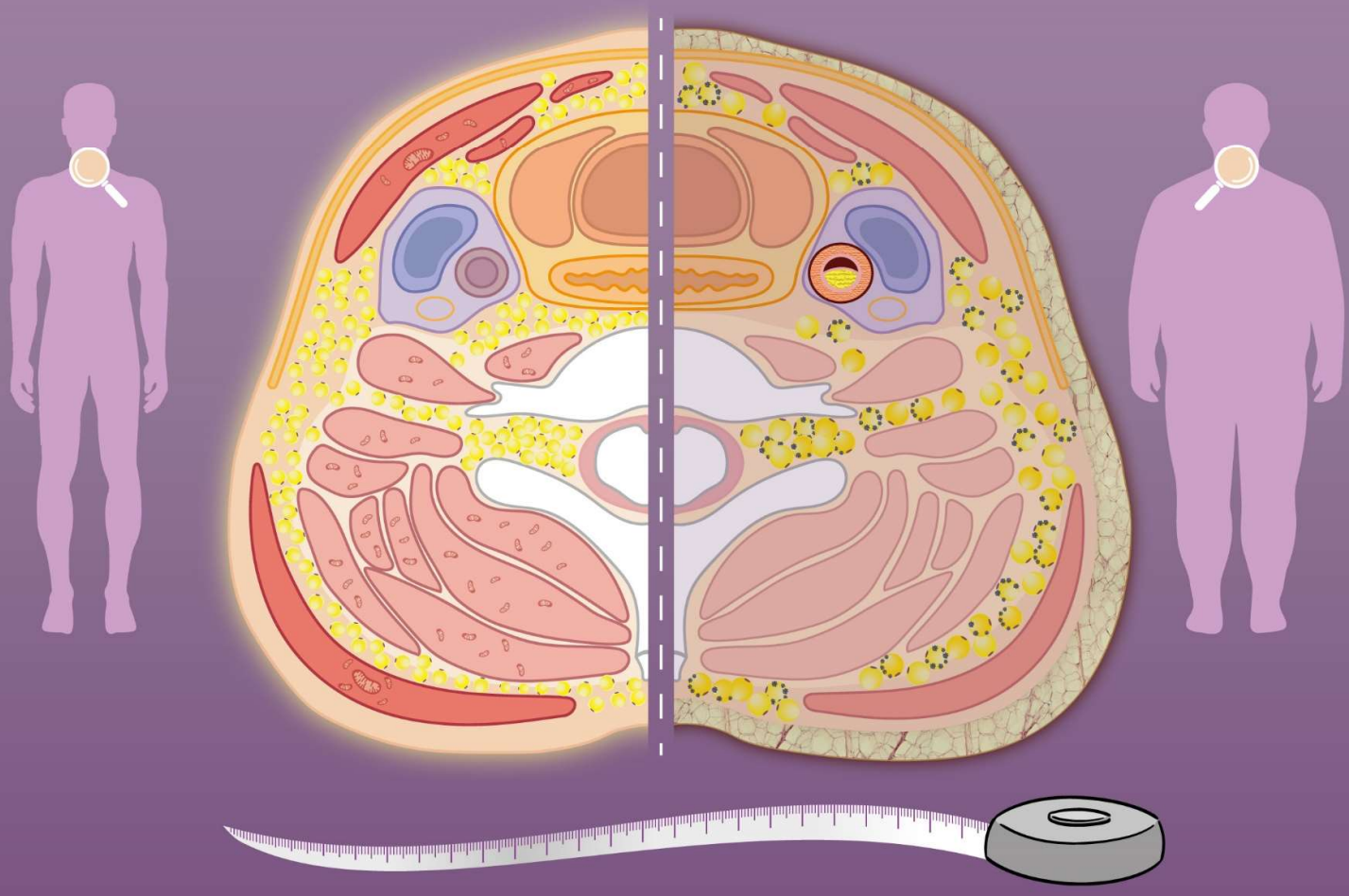
DOCTORAL PROGRAMME IN BIOMEDICINE

NECK ADIPOSE TISSUE AND CIRCUMFERENCE AS PREDICTORS OF CARDIOMETABOLIC RISK IN SEDENTARY ADULTS

María José Arias Téllez



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María José Arias Téllez

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Granada, 01 Julio de 2020

El doctorando María José Arias Téllez ha realizado la presente Tesis Doctoral como beneficiario de una beca de Doctorado de la Fundación Carolina, Convenio 2016.

A mi hermano Miguel, en honor a la vida

A mi familia, por enseñarme el valor de la humildad, honestidad y perseverancia

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01

PROLOGUE

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PROLOGUE

RESEARCH PROJECTS AND FUNDING

The present International Doctoral Thesis was carried out under the framework of the ACTIBATE study (https://clinicaltrials.gov/ct2/show/NCT02365129) which was funded by the following organizations:

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ABREVIATION

ACTIBATE: : Activating brown adipose tissue through exercise.

BAT: Brown adipose tissue.

BIA: Bioelectrical impedance analysis.

BMI: Body mass index.

DXA Scan: Dual-Energy X-Ray Absorptiometry Scan.

LMI: Lean mass index.

LPA: Light physical activity.

FMI: Fat mass index.

CMR: Cardiometabolic risk.

CT: Computed tomography

MPA: Moderate physical activity.

MVPA: Moderate- vigorous physical activity.

MVPA10min: Moderate- vigorous physical activity in bouts 10 minutes.

NAT: Neck adipose tissue.

NC: Neck circumference.

PA: Physical activity.

Tight IMAT: Intermuscular adipose tissue in the tight.

VAT: Visceral adipose tissue.

VPA: Vigorous physical activity.

WAT: White adipose tissue.

Waist/hip: ratio waist/hip.

Waist/height: ratio waist/height.

WC: Waist circumference.

DEFINITIONS OF THE MAIN TERMS USED IN THIS DOCTORAL THESIS

Adipose tissue. Specialized connective tissue composed of fat cells (adipocytes). It is the site of stored fats, usually in the form of triglycerides. In mammals, there are two types of adipose tissue, the white fat and the brown fat. Their relative distributions vary in different species with most adipose tissue being white (Mesh term).

Adipocyte. Cells in the body that store fats, usually in the form of triglycerides. White adipocytes are the predominant type and found mostly in the abdominal cavity and subcutaneous tissue. Brown adipocytes are thermogenic cells that can be found in newborns of some species and hibernating mammals (Mesh term).

Anthropometry. The technique that deals with the measurement of the size, weight, and proportions of the human or other primate body (Mesh term).

Body composition. The relative amounts of various components in the body, such as percentage of body fat (Mesh term).

Brown adipose tissue. A thermogenic form of adipose tissue composed of brown adipocytes. It is found in newborns of many species including humans, and in hibernating mammals. Brown fat is richly vascularized, innervated, and densely packed with mitochondria which can generate heat directly from the stored lipids (Mesh term).

Body mass index. An indicator of body density as determined by the relationship of body weight to body height. BMI=weight (kg)/height squared (m2). BMI correlates with body fat (adipose tissue). Their relationship varies with age and gender. For adults, BMI falls into these categories: below 18.5 (underweight); 18.5-24.9 (normal); 25.0-29.9 (overweight); 30.0 and above (obese). (National Center for Health Statistics, Centers for Disease Control and Prevention), (Mesh term).

Brown Adipose tissue. A thermogenic form of adipose tissue composed of brown adipocytes. It is found in new-borns of many species including humans, and in hibernating mammals. Brown fat is richly vascularized, innervated, and densely packed with mitochondria which can generate heat directly from the stored lipids (Mesh term).

Cardiometabolic risk. Refers to risk factors that increase the likelihood of experiencing vascular events or developing diabetes. This concept encompasses traditional risk factors included in risk calculators, such as hypertension, dyslipidemia, and smoking, as well as emerging risk factors, such as abdominal obesity, inflammatory profile, and ethnicity (1).

Dual-energy x-ray absorptiometry scan. A non-invasive method for assessing body composition. It is based on the differential absorption of x-rays (or gamma rays) by different tissues such as bone, fat and other soft tissues. the source of (x-ray or gamma-ray) photon beam is generated either from radioisotopes such as gadolinium 153, iodine 125, or americanium 241 which emit gamma rays in the appropriate range; or from an x-ray tube which produces x-rays in the desired range. It is primarily used for quantitating bone mineral content, especially for the diagnosis of osteoporosis, and also in measuring bone mineralization (mesh term).

Exercise. Physical activity which is usually regular and done with the intention of improving or maintaining physical fitness or health. Contrast with physical exertion which is concerned largely with the physiologic and metabolic response to energy expenditure (mesh term).

Fat mass. Term derived of molecular analyze of body composition. Is synonymous of triglycerides and subcategory of total lipid (2).

Hypertrophy of adipocyte. Increased of adipocyte size under excess energy conditions (3).

Hyperplasia. Expanded of Adipose tissue trough increase of the number (3).

Interleukins. Soluble factors which stimulate growth-related activities of leukocytes as well as other cell types. They enhance cell proliferation and differentiation, DNA synthesis, secretion of other biologically active molecules and responses to immune and inflammatory stimuli. (Mesh term).

Inflammation. A pathological process characterized by injury or destruction of tissues caused by a variety of cytologic and chemical reactions. It is usually manifested by typical signs of pain, heat, redness, swelling, and loss of function (Mesh term).

Insulin Resistance. Diminished effectiveness of insulin in lowering blood sugar levels: requiring the use of 200 units or more of insulin per day to prevent hyperglycemia or ketosis (Mesh term).

Lipolysis. The metabolic process of breaking down lipids to release free fatty acids, the major oxidative fuel for the body. Lipolysis may involve dietary lipids in the digestive tract, circulating lipids in the blood, and stored lipids in the adipose tissue or the liver. A number of enzymes are involved in such lipid hydrolysis, such as lipase and lipoprotein lipase from various tissues (Mesh term).

Lipogenesis. De novo fat synthesis in the body. This includes the synthetic processes of fatty acids and subsequent triglycerides in the liver and the adipose tissue. Lipogenesis is regulated by numerous factors, including nutritional, hormonal, and genetic elements (Mesh term).

Intermuscular neck adipose tissue. Fat between the sternocleidomastoid, scalene and trapezius muscles and that has been separated from the superficial compartment by the deep cervical fascia in the neck (4).

Lean body mass. Term derived of molecular analyze of body composition. Unlike of fat free mass, include essential lipid (2).

Lower body fat. Gluteal and femoral fat localized in the lower recon of the body (5).

Macrophages. The relatively long-lived phagocytic cell of mammalian tissues that are derived from blood monocytes. Main types are peritoneal macrophages; alveolar macrophages; histiocytes; kupffer cells of the liver; and osteoclasts. They may further differentiate within chronic inflammatory lesions to epithelioid cells or may fuse to form foreign body giant cells or langhans giant cells. (From The Dictionary of Cell Biology, Lackie and Dow, 3rd ed.) (Mesh term).

Neck circumference. Perimeter measured around of neck at level of cricotiroídeo cartilage (6).

Neck adipose tissue. Adipose tissue accumulation in the neck distributed in different compartments (4).

Obesity. A status with body weight that is grossly above the acceptable or desirable weight, usually due to accumulation of excess fats in the body. The standards may vary with age, sex, genetic or cultural background. In the body mass index, a BMI greater than 30.0 kg/m2 is considered obese, and a BMI greater than 40.0 kg/m2 is considered morbidly obese (morbid obesity), (Mesh term).

Perivertebral neck adipose tissue. Fat interspersed between muscles surrounding the cervical spine in the neck (4).

Upper body fat. Subcutaneous and visceral depots localized in the upper region of the body (5).

Physical activity. Defined as any bodily movement produced by skeletal muscle that requires energy expenditure. It can be undertaken in many different ways: walking, cycling, sports and active form of recreation (ex. dance, yoga, tai chi).

Also, can be undertaken as part of work or domestic task around of home. However, all the form of PA provided healthy benefits if undertaken regularly and of sufficient duration and intensity (7).

Sexual dimorphism. Sex- dependent differences related with distribution and function of adipose tissue (5).

Sedentary behavior. Defined as any waking behavior characterized for an energy expenditure ≤ 1.5 metabolic equivalents such as sitting, reclining or lying down (7).

Subcutaneous Fat. Fatty tissue under the skin throughout the body (Mesh term).

Subcutaneous neck adipose tissue. Adipose tissue that lies between the skin and the deep cervical fascia of the neck (4).

Tumor Necrosis Factor-alpha (TNF-alfa). Serum glycoprotein produced by activated macrophages and other mammalian mononuclear leukocytes. It has necrotizing activity against tumor cell lines and increases ability to reject tumor transplants. Also known as TNF-alpha, it is only 30% homologous to TNF-beta (lymphotoxin), but they share TNF receptors (Mesh term).

Tomography axial computerized. X-ray image-detecting devices that make a focused image of body structures lying in a predetermined plane from which more complex images are computed (Mesh term).

Thig Intermuscular adipose tissue. Ectopic adiposity characterized by the storage of adipose tissue in the thigh (non-subcutaneous site) associated with metabolic and cardiovascular disorders (8).

Visceral adipose tissue. Fatty tissue inside the abdominal cavity, including visceral fat and retroperitoneal fat. It is the most metabolically active fat in the body and easily accessible for lipolysis. Increased visceral fat is associated with metabolic complications of obesity (Mesh term).

Waist circumference. The measurement around the body at the level of the abdomen and just above the hip bone. The measurement is usually taken immediately after exhalation (Mesh term).

White adipose tissue. Fatty tissue composed of white adipocytes and generally found directly under the skin (subcutaneous fat) and around the internal organs (abdominal fat). It has less vascularization and less coloration than the brown fat. White fat provides heat insulation, mechanical cushion, and source of energy (Mesh term).

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ABSTRACT

Background: Upper body subcutaneous fat is related to cardiometabolic risk (CMR) factors, independently of visceral adipose tissue (VAT), suggesting that other fat deposits beyond that of VAT, could be explaining this additional risk.
Aims: To study the association between neck adipose tissue (NAT) and neck circumference (NC) with CMR, inflammatory factors, sedentary time, physical activity (PA), and examine the dose-response effect of a concurrent exercise intervention of six months on neck measurements in sedentary adults.
Methods: This doctoral thesis was structured in 4 cross-sectional studies (**studies 1, 2, 3 and 4**) and 1 randomized controlled trial (**study 5**). Sedentary young Spanish adults aged 18 to 25 years old (**studies 1, 3, 4 and 5**) were included. The participants were randomized into three groups: control, moderate-intensity and vigorous-intensity exercise in the ACTIBATE project (Clinical Trial Registration: NCT002365129). The NAT was estimated using positron emission tomography combined with computed tomography. Compartmental (subcutaneous, intermuscular and perivertebral) and total NAT volumes were determined, and NC was measured. Additionally, we used data of another study with available information on NC and intermuscular adipose tissue content in the thigh of premenopausal women with overweight and obesity (n=142, Clinical Trial Registration: NCT00513084, **study 2**).
Results: NC was positively associated with markers of body composition in women and men young ($P \leq 0.002$) (**study 1**), and with adipose tissue content in thigh skeletal muscle in premenopausal women ($P \leq 0.001$) (**study 2**). In addition (**study 3**), NAT was directly associated with CMR and inflammatory status (independently of VAT), ($P \leq 0.001$) and inversely associated with PA levels, mainly in men (**study 4**). Lastly, moderate and vigorous-intensity exercise decreased significantly, 1.01 and 0.77 centimetres of NC, respectively (**study 5**).
Conclusions: The present doctoral thesis provides novel information about the relationship between NAT depots with overall and central adiposity, CMR factors, inflammatory status, sedentary behaviour and PA levels. It also shows the impact of an exercise intervention on neck measurements. In addition, these findings highlight the utility of NC as a subrogate indicator of NAT estimation and ectopic fat deposition in the thigh skeletal muscle in overweight and obese premenopausal women.

RESUMEN

Antecedentes: La grasa subcutánea del tronco superior es relacionada con factores de riego cardiometabólico (RCM), independientemente del tejido adiposo visceral (TAV), sugiriendo que otros depósitos de grasa más allá del TAV, podrían estar explicando este riesgo adicional.

Objetivos: Estudiar la asociación entre el tejido adiposo del cuello (TA cuello) y la circunferencia del cuello (CC) con RCM, factores inflamatorios, tiempo de sedentarismo, actividad física (AP), y examinar el efecto dosis-respuesta de una intervención de ejercicio concurrente de seis meses sobre mediciones de cuello en adultos sedentarios.

Métodos: Esta tesis doctoral se estructuró en 4 estudios transversales (e**studios 1, 2, 3 y 4**) y 1 ensayo aleatorio controlado (**estudio 5**). Adultos jóvenes españoles sedentarios entre 18 y 25 años (**estudios 1, 3, 4 y 5**) fueron incluidos. Los participantes fueron aleatorizados en tres grupos en el proyecto ACTIBATE (Registro de ensayos clínicos: NCT002365129): grupo control, grupo de ejercicio de intensidad moderada y grupo de ejercicio de intensidad vigorosa. El TA cuello fue estimado mediante tomografía por emisión de positrones combinada con tomografía computarizada. Los volúmenes de TA cuello compartimental (subcutáneo, intermuscular y perivertebral), TA cuello total, y CC fueron medidos. Adicionalmente, utilizamos datos de otro estudio con información disponible sobre CC y contenido de tejido adiposo intermuscular en el muslo de mujeres pre-menopáusicas con sobrepeso y obesidad (n = 142, Registro de ensayos clínicos: NCT00513084, **estudio 2**).

Resultados: CC fue asociado positivamente con marcadores de composición corporal en mujeres y hombres jóvenes ($P \leq 0.002$) (**estudio 1**), y con contenido de tejido adiposo en el músculo esquelético del muslo en mujeres pre-menopáusicas ($P \leq 0.001$) (**estudio 2**). Además (**estudio 3**), TA cuello fue directamente asociado con RCM y estado inflamatorio (independientemente del TAV), ($P \leq 0.001$) e inversamente asociado con niveles AP, principalmente en hombres (**estudio 4**). Por último, el ejercicio de intensidad moderada y vigorosa disminuyó significativamente la CC en 1.01 y 0.77 centímetros, respectivamente (**estudio 5**).

Conclusiones: La presente tesis doctoral proporciona novedosa información sobre la relación entre los depósitos TA cuello con adiposidad general y central, factores de RCM, estado inflamatorio, comportamiento sedentario y niveles de AP. También muestra el impacto de una intervención de ejercicio sobre las mediciones de cuello. Adicionalmente, estos hallazgos resaltan la utilidad de la CC como indicador subrogado de la estimación de TA cuello de adultos jóvenes y del depósito de grasa ectópica en el músculo esquelético del muslo de mujeres pre-menopáusicas con sobrepeso y obesidad.

02

GENERAL INTRODUCTION

Tejido Adiposo: importancia en salud y enfermedad
Métodos de evaluación de la composición corporal
Perímetro de cuello
Tejido adiposo del cuello

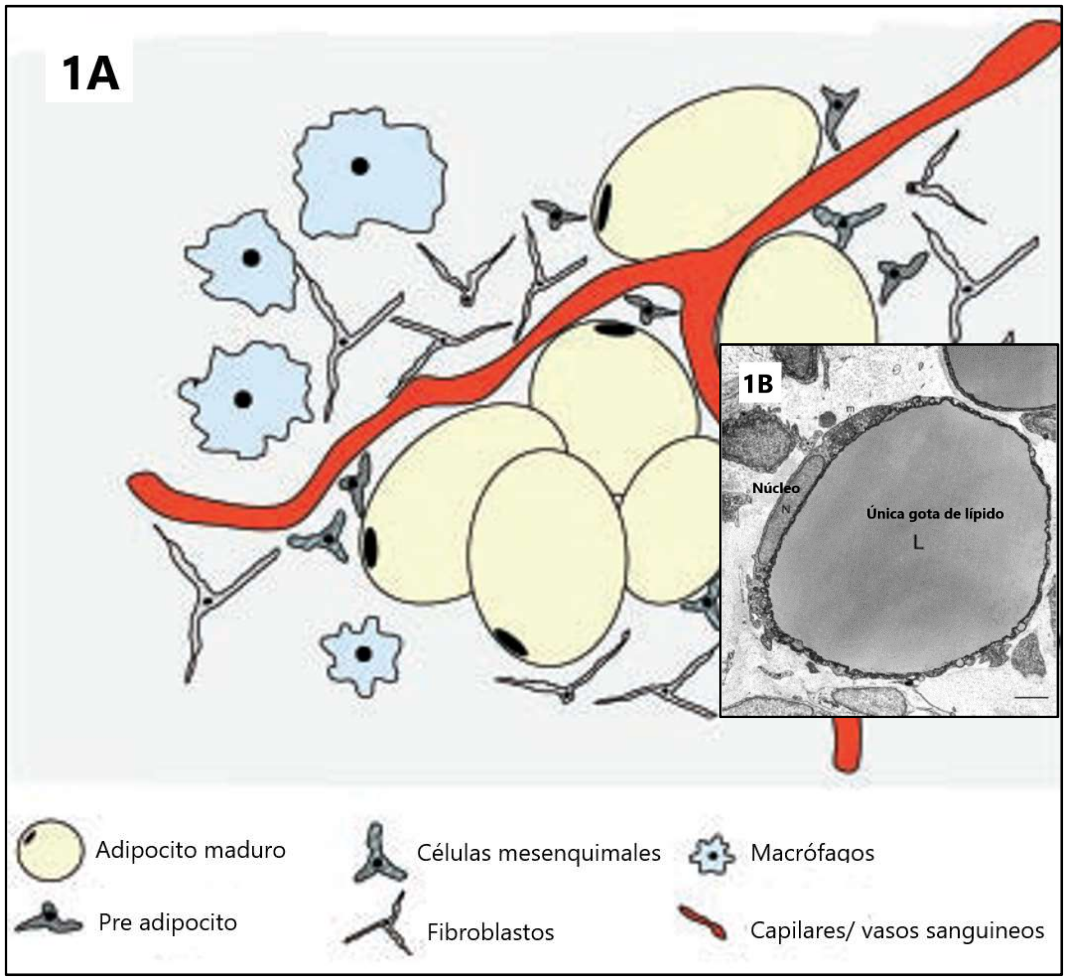
INTRODUCCION GENERAL

La obesidad es un problema público en todo el mundo, atribuyéndole más de 4 millones de muertos por año (1). Recientemente, un tercio de la población mundial ha sido clasificada con sobrepeso u obesidad, afectando a personas de todas las edades y ambos sexos (2). La ausencia de soluciones efectivas para la reducción de peso a largo plazo ha aumentado la magnitud de este problema. En los últimos años, los esfuerzos se han orientado a incrementar los hallazgos que faciliten una mejor comprensión de la distribución de los depósitos de grasa (independiente de la obesidad general), y su efecto sobre factores de riesgo cardio metabólico (RCM), que puedan generar nuevas herramientas en el diagnóstico y manejo de esta enfermedad.

i) Tejido Adiposo: importancia en salud y enfermedad

El tejido adiposo es un órgano endocrino que se caracteriza por realizar funciones de lipogénesis, lipolisis, captación de glucosa y secreción de adipoquinas (3). Sus distintos depósitos anatómicos a lo largo del cuerpo, están constituido por tres tipos diferentes de adipocitos: blanco, beige y marrón, los cuales se encuentran integrados mediante células del estroma vascular (soporte-vascularización-inervación, pre adipocitos y células del sistema inmune) con una capacidad de crecimiento normalmente entre 20-200mm (4; 5) (ver, figura 1A). Los adipocitos blancos maduros (totalmente diferenciados) tienen forma esférica y una única gota de lípido rodeado por un borde reducido de citoplasma (6). Por consecuencia, el núcleo y todos los orgánelos (mitocondrias, retículo endoplásmico y lisosomas) son empujados a la periferia de la célula (ver, figura 1B).

Figura 1. (1A) Estructura del tejido adiposo (adipocito maduro + estroma vascular), (1B) Características del adipocito blanco.



Figuras extraídas de Armani y cols, J. 2010 y Giordano A. y cols 2014. Adaptada por Arias MJ.

El tejido adiposo blanco (TAB) es un órgano complejo que no solo tiene funciones de almacenamiento y utilización de energía (7). Un desequilibrio entre ingesta y gasto de energético da como resultado una condición de incremento de peso, TAB y como consecuencia obesidad (8). Para satisfacer la sobrecarga de energía el TAB debe realizar múltiples mecanismos celulares de remodelación estructural con el objetivo de optimizar su expansión, requiriendo de la coordinación de dos procesos conocidos como hiperplasia (aumento del número de adipocitos) e hipertrofia (aumento del tamaño) (9). Es así como en condiciones de excesiva y

limitada expansión el adipocito se hipertrofia, remodelando el contenido de su matriz extracelular (principalmente de colágeno IV), aumentando la producción de quimioquinas que atraen macrófagos (de tipo M1), inhibiendo el proceso de angiogénesis (formación de nuevos vasos sanguíneos) que favorece la hipoxia y generando un estado de inflamación crónica. De esta manera, los macrófagos invaden el TAB, el cual comienza a secretar citoquinas pro-inflamatorias (como TNF alfa, IL-6, IL-1β) que regulan mecanismos en otros tejidos (entre ellos el bloqueo del reclutamiento de pre adipocitos), (7; 10; 11) (ver, figura 2). Adicionalmente, un incremento del tamaño del adipocito, repercute en un ambiente metabólico desfavorable promoviendo complicaciones metabólicas asociadas a la obesidad (9). El excesivo almacenamiento de triglicéridos al interior del adipocito hipertrófico aumenta la cantidad de diacilglicéridos y ceramidas, alterando la cascada de señalización de la insulina (IRS-1 y Akt1), disminuyendo la translocación de Glut 4 e impidiendo la entrada de glucosa al interior de la célula (12). Como consecuencia, los niveles de glucosa e insulina incrementan de manera sostenida, pudiendo generar a largo plazo necrosis de los islotes B de células pancreáticas y diabetes mellitus 2 (13; 14). Adicionalmente, la actividad lipolitica de los adipocitos se incrementa, consecuencia de un déficit de glucosa generado por la resistencia a la insulina de adipocitos y grandes grupos musculares. Este fenómeno, sería parte de la infiltración de ácidos grasos en tejidos ectópicos, tales como hígado (esteatosis hepática) (15) y músculo esquelético (16).

Figura 2. Circulo entre excesiva ingesta energética, hipertrofia del tejido adiposo, infiltración de macrófagos y liberación de citoquinas pro inflamatorias en condiciones de obesidad.

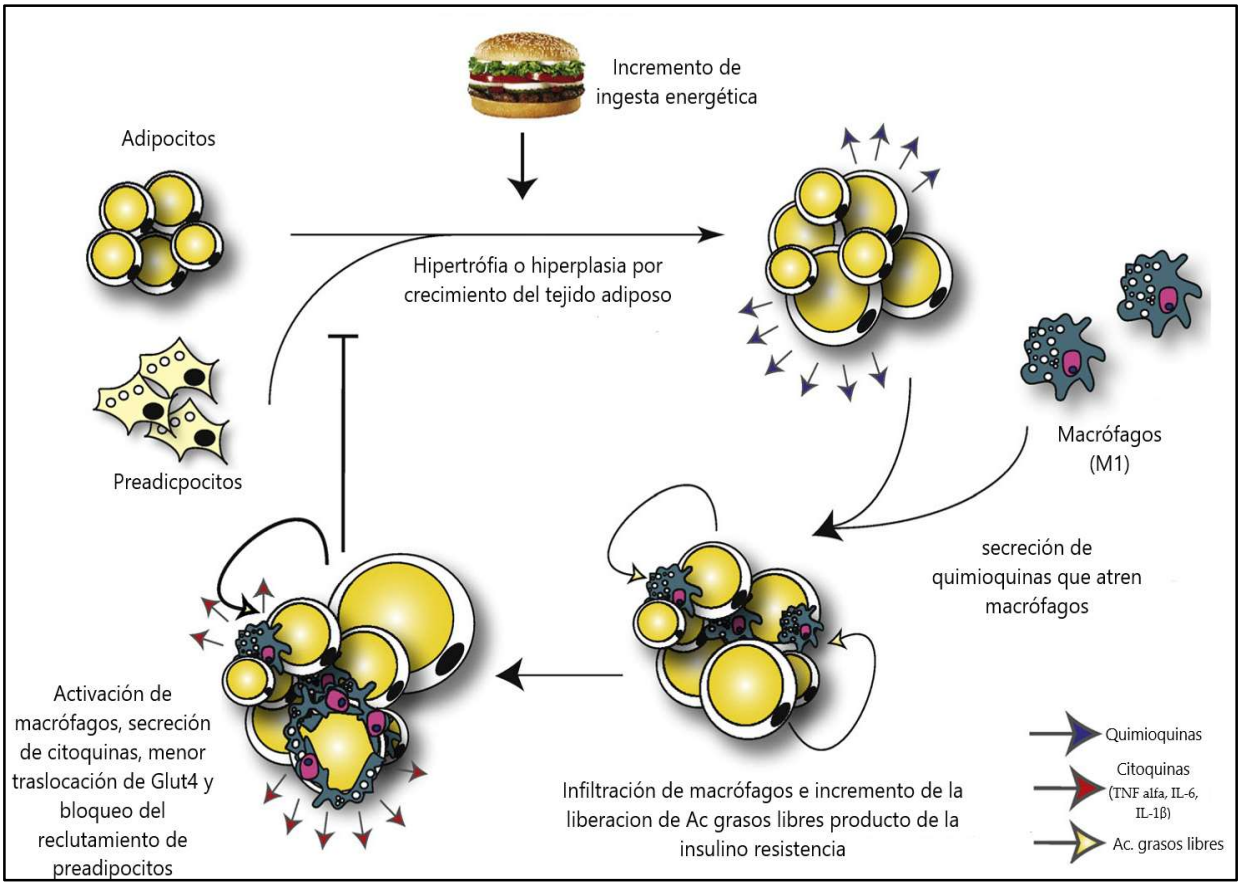


Figura extraída de Virtue, S y cols 2010 y adaptada por Arias MJ.

Cada depósito individual de grasa es complejo y funcionalmente diferente. La distribución y características del TAB en el cuerpo también han sido relacionadas con salud y enfermedad (9). Curiosamente, la grasa de la parte superior del cuerpo (abdominal visceral y subcutánea) ha sido asociada con complicaciones desfavorables de la obesidad, mientras que la grasa de la parte inferior del cuerpo (glúteo-femoral) ha tenido un perfil protector (17). En esta línea, estudios más recientes han mostrado que los adipocitos del tejido adiposo visceral y subcutáneo, serían heterogéneos debido a que tendrían distintos orígenes genéticos (18; 19). Mientras el adipocito visceral se caracteriza por tener un

mayor tamaño con mayor expresión de hormona sensible a lipasa (HSL), perilipina (20) y receptores beta-adrenérgicos (favorecedores de lipolisis) (17), los adipocitos subcutáneos se caracterizan por ser más pequeños y tener mayor número de receptores alfa- adrenérgicos (anti-lipolíticos) (21; 22). Adicionalmente, estas diferencias influirían sobre la expresión y secreción de factores endocrinos. De hecho, una mayor secreción de IL-6 y PAI-1 (inhibidor del activador de plasminógeno 1) ha sido relativamente asociada al tejido adiposo visceral, mientras que la secreción de leptina y adiponectina al tejido adiposo subcutáneo (23). En este contexto, que los hombres tengan una mayor acumulación de grasa a nivel central a diferencia de las mujeres que lo hacen a nivel glúteo-femoral, destaca la importancia de comprender mejor el mecanismo biológico de las diferencia de sexo y género en condiciones de obesidad (24; 25).

Tradicionalmente, diferencias de secreciones gonadales, comúnmente referidas a las hormonas sexuales entre hombres y mujeres (determinadas por los cromosomas sexuales XX e XY, respectivamente) han sido relacionados con diferencias en el metabolismo de los lípidos (26). Por ejemplo, mientras las mujeres han desarrollado mecanismos específicos para favorecer el almacenamiento de tejido adiposo, la movilización de las reservas de grasa tiende a ser más eficiente en los hombres (24). Un gran número de evidencia ha mostrado que los andrógenos son moduladores críticos de la distribución de grasa corporal tanto en hombres como en mujeres (27). En esta línea, previos estudios han reportado la importancia de las hormonas gonadales en mujeres pre y post menopaúsicas, encontrando en estas últimas, que la disminución de los niveles de estrógenos y aumento de andrógenos (testosterona), repercuten en los cambios de distribución del depósito de tejido adiposo (principalmente visceral) (28; 29). Similares resultados han sido observados en mujeres con síndrome de ovario poliquístico, donde un incremento de testosterona plasmática ha sido

asociado a un mayor tamaño de los adipocitos, aumento de acumulación de grasa a nivel central e incremento del RCM (30-32).

En el caso de los hombres, los niveles de testosterona, también influyen sobre la distribución de grasas y el RCM (27; 33). Estudios transversales y longitudinales sobre andrógenos y distribución de grasa corporal han sido consistentes en mostrar que la asociación entre obesidad central y síndrome metabólico son proporcionalmente asociadas con niveles más bajos testosterona total (34; 35). Por lo tanto, una mayor liberación de ácidos grasos al plasma, característico los adipocitos más lipolíticos, podrían ser parte de algunos de los mecanismos que explicarían la estrecha relación entre concentración de andrógenos, depósito de tejido adiposo central y RCM, predominantemente mayor en mujeres postmenopáusicas, síndrome de ovario poliquístico y hombres.

ii) Métodos de evaluación de la composición corporal

La incidencia de obesidad en países en desarrollo y vías de desarrollo, se ha convertido en una epidemia, impactando dramáticamente sobre la salud metabólica e insulino sensibilidad (36). La necesidad de conocer los pro y contra de distintos métodos de la composición corporal a la hora de diagnosticar y monitorear esta patología crónica, es imprescindible (37).

Para un mejor entendimiento e interpretación de los distintos métodos de composición corporal, es necesario conocer conceptos que involucran**: a) nivel de análisis del cuerpo, b) tipo de técnica utilizada y c) número de componentes de la técnica utilizada.**

a. Nivel de análisis del cuerpo:

El cuerpo humano puede cuantificarse en varios niveles, dependiendo del interés clínico. Los métodos de evaluación de la composición corporal pueden ser categorizados (38; 39) en cinco tipos (ver figura 1) de acuerdo a los distintos niveles de análisis:

- I. Nivel atómico: formado por átomos (oxígeno, carbono e hidrógeno) y elementos (calcio y fósforo) que constituyen el 98% de cuerpo completo. Dentro de ellos el 60% es oxígeno.

- II. Nivel molecular: constituido por agua (60%), lípidos, proteínas y otros (glicógeno y minerales). Controversialmente, el término "lípido" es frecuentemente intercambiable con el término "grasa", lo cual puede conducir a malas interpretaciones de los principales modelos de composición corporal. La definición tradicional de "lípido" se refiere a un grupo de compuestos químicos insolubles en agua y muy solubles en disolventes orgánicos como éter dietílico, benceno y cloroformo (40). Cerca de 50 tipos diferentes lípidos son reconocidos en humanos, los cuales son divididos en cinco subcategorías: 1) lípidos simples (incluidos triglicéridos y ceras), 2) lípidos compuestos (por ejemplo, fosfolípidos y esfingolípidos), 3) esteroides, 4) ácidos grasos y 5) terpenos. En cambio la grasa es sinónimo de triglicérido (3 ácidos grasos + 1 glicerol) y por ende una subcategoría de los lípidos (40).

- III. Nivel celular: Aunque el cuerpo humano se puede dividir en diferentes componentes a nivel molecular, es el ensamblaje de estos componentes los que crean el organismo vivo. A nivel celular, el cuerpo humano está compuesto de células, fluido extracelular y sólidos extracelulares.

- IV. Nivel de tejidos. Los tres componentes del nivel celular, se organizan en tejidos, órganos y sistemas, creando el cuarto nivel composición corporal.

- V. Nivel de cuerpo completo. El nivel de composición corporal de todo el cuerpo otorga al cuerpo tamaño, forma y características físicas exteriores, cuantificables a través de: peso corporal total, estatura, índices, circunferencias, longitudes, diámetros de huesos, pliegues cutáneos, áreas superficiales, volumen y densidad corporal.

Figura 3. Niveles de composición corporal.

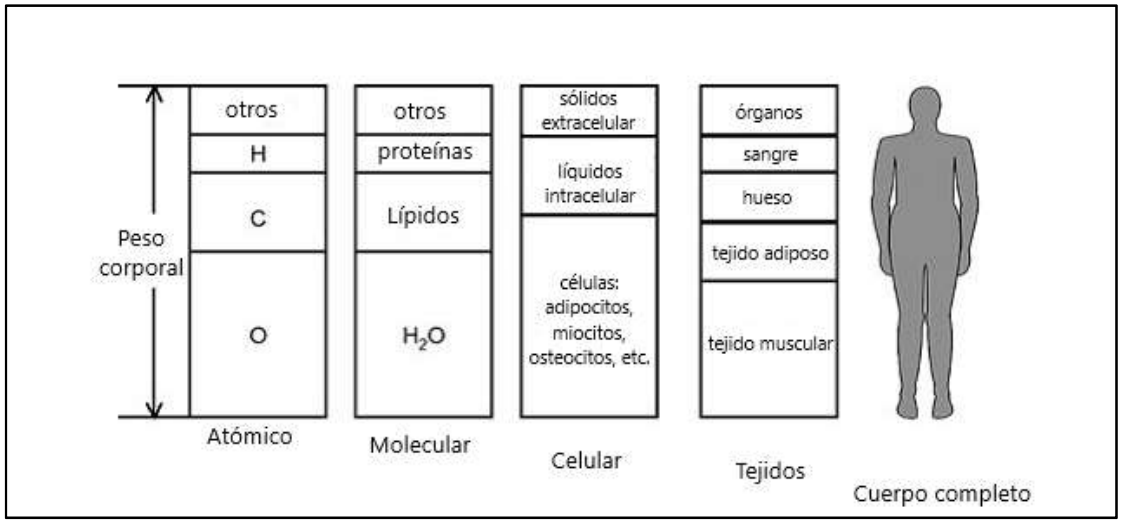


Figura extraída de Adaptado de Wang et al., 1992 y Marie Ø. Fosbøl y cols y adaptada por Arias MJ.

b. Número de componentes:

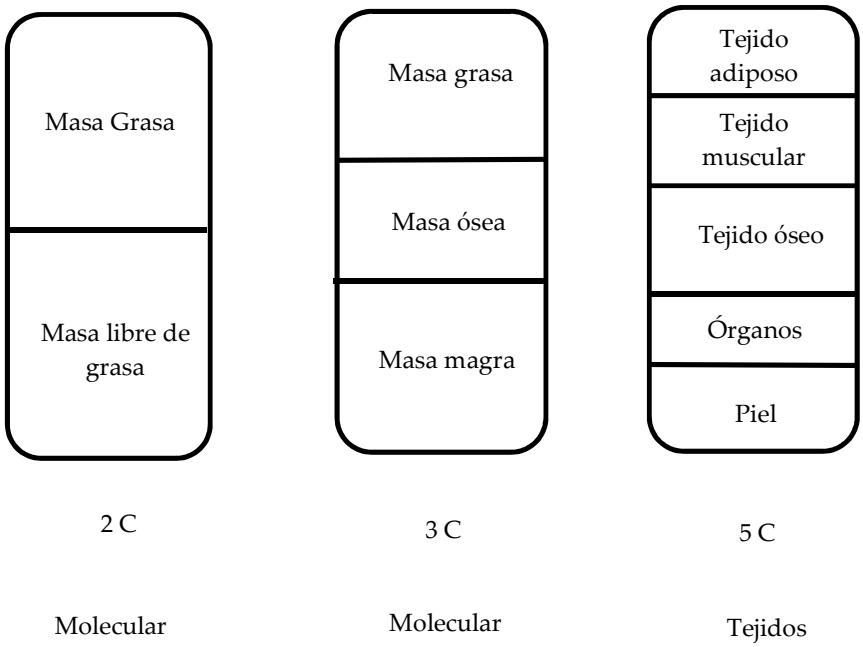
Según el componente químico del cuerpo, diferentes modelos de composición corporal han sido desarrollados (ver figura 4). Los más utilizados, son descritos a continuación:

- Modelo de 2 componentes: Originalmente desarrollado por Alberto R. Benke (1942), el cual separa al cuerpo humano en dos componentes: masa grasa y masa libre de grasa, asumiendo según el principio de Arquímedes, densidades constantes de 0.900 g / cm^3 y 1.095 g / cm^3, respectivamente (41). Años después Siri (1961) (42), mediante la técnica de hidrodensitometría, desarrolla un modelo de densidad corporal, muy popular para la estimación de porcentaje de grasa corporal, asumiendo constates de 0.900 g/cm^3 and 1.100 g/cm^3 a 37º C para masa grasa y masa libre de grasa, respectivamente. Similarmente, años después, Brozek (1963) también propone una ecuación para estimación de grasa corporal, pero asumiendo densidades constantes de 0.9007 g / cm^3 y 1.100 g / cm^3 a 36º C, para masa grasa y masa libre de grasa respectivamente (43).

- Modelo de 3 componentes: Desarrollado por Siri después de identificar una variación del 2% de hidratación del componente masa libre de grasa del modelo de hidrodensitometría de 2 componentes. Como consecuencia asume una densidad de masa residual (1.565 g / cm^3), la cual refleja la densidad de proteínas (1.34 g / cm^3) y minerales (3.00 g / cm^3) (44), antes no considerada. Actualmente, en técnicas avanzadas (DXA), este modelo divide al cuerpo en masa grasa, masa magra suave (agua, proteínas, glicógeno y masa mineral no ósea) (45) y masa ósea (46).

- Modelo multicomponentes: Se encuentran el modelo de 4 componentes (masa grasa, agua corporal, masa ósea y masa residual) (46), el modelo antropométrico de 5 componentes (tejido adiposo, tejido muscular, tejido óseo, tejido residual y piel) de fraccionamiento de masas de Devora Kerr (1988) (47), o aquellos utilizados por técnicas avanzadas como tomografía axial computarizada o imágenes de resonancia magnética.

Figura 4. Componentes de análisis de diferentes niveles de composición corporal.



c. Tipo de técnicas de análisis de composición corporal:

Adicionalmente, los distintos métodos pueden ser clasificados según el tipo técnica de análisis utilizada (37; 48), en:

- Métodos directos: Donde se encuentra la única e inigualable técnica de disección cadavérica o patrón oro. La disección de los cadáveres de Bruselas, es el más famoso, debido a la validación de la técnica antropométrica (Débora Kerr) de estimación de tejidos de 5 componentes (49; 50).

- Métodos Indirectos o de Referencia: Donde se encuentran principalmente los métodos de laboratorio como absorciometría de rayos X de energía dual (DXA), tomografía computarizada y resonancia magnética, los cuales han sido definidos como técnicas gold estándar para la validación de diferentes métodos doblemente indirectos (51). Adicionalmente las formulas antropométricas de estimación de tejidos, derivadas del estudio de cadáveres de Bruselas, también son consideradas un método de tipo indirecto (47).

- Métodos doblemente-indirectos o de campo: Ampliamente utilizados a nivel de campo debido a su bajo costo (51). Acá encontramos ecuaciones antropométricas de estimación de porcentaje de masa grasa mediante densidad (Siri & Durnin) (42; 52) y análisis de bioimpedanciometría (BIA) que derivan tal de la técnica de hidrodensitometría (53).

Las características, ventajas y limitaciones de los principales métodos de análisis de composición corporal son resumidas en tabla 1.

Tabla 1. Características, ventajas y limitaciones de los diferentes métodos de composición corporal.

Método	Características	Nivel de análisis	Nº de componentes	Clasificación de la técnica	Ventajas	
Fórmulas antropométricas Siri & Durnin.	Método de estimación de composición corporal, a través de la medición de 4 pliegues cutáneos (bicipital, tricipital, subescapular, suprailiaco) (42).	Molecular	2C	Doblemente indirecta o de campo	- Bajo costo. - Rápido. - Transportable. - Exitoso para detectar cambios de composición corporal.	- Requier - Invalido
Bioimpedanciometría.	Método de estimación de composición corporal, el cual interpreta el paso de una corriente eléctrica de bajo voltaje a través de los tejidos a través de impedancia (54).	Molecular	2 C	Doblemente indirecta o de campo	- Bajo costo. - Rápido. - Transportable.	Posibles fu - Deshidr - Estado - Temper - Asimetr - Posición
Absorciometría dual de ratos X.	Estimación de la composición corporal, mediante el uso de un escáner que mide en dos tipos diferentes de energía, la atenuación de fotones emitidos por ondas de baja radiación (55).	Molecular	3 C	Indirecta o de laboratorio	- Preciso. - Exacto. - Rápido. - No invasivo. - Entrega información acerca de grasa total y grasa regional.	- No tran - Mayor - Exposic - Diferen máquin marcas) - Dificult - El grado estimac

Tomografía Axial computarizada.	Técnica de análisis de imágenes mediante la segmentación de tejidos, basado en la atenuación especifica de señal (Hounsfield Units →HU) (44).	Tejidos	Multi-componentes	Indirecta o de laboratorio	- Alta precisión. - Valoración de grasa subcutánea y grasa visceral, como también grasa pericárdica, grasa intratorácica y grasa epicárdica. - Útil para distinguir infiltración de grasa en músculo e hígado.	- No tran - Alto cos - Exposic - Dificult obesos
Resonancia magnética.	Técnica caracterizada por cuantificar mediante vóxel (del inglés volumetric pixel) la cantidad y distribución de tejido adiposo (subcutáneo, visceral, intramuscular), músculo esquelético, edema y diversos órganos (hígado, riñones, corazón, bazo, páncreas) (56).	Tejidos	Multi-componentes	Indirecta o de laboratorio	- Alta precisión - Mejor resolución de imágenes. - Sin exposición a la radiación. - A diferencia del DXA no se ve influenciada por el grado de hidratación. - Debido a la ausencia de radiación, puede usarse en niños y adolescentes.	- Muy ca - No tran - Mayor t - Necesid - Dificult obesos.
Fórmulas antropométricas Debora Kerr.	Método antropométrico de fraccionamiento de masas, construido en base a evidencia atómica de cadáveres de 1669 entre 6-77 años (47).	Tejidos	5C	Indirecta o de laboratorio	- Bajo costo. - Transportable. - No invasivo - Estimación de tejido adiposo, muscular y óseo, mediante ecuaciones.	- Requier - Requier tiempo técnica

2C: 2 componentes; 3 C: 3 componentes; 4C: 4 componentes, 5 C: componentes.

iii) Perímetro de cuello

El IMC es el marcador más utilizado para categorizar a las personas según su estado de peso corporal en bajo-peso, normo-peso, sobrepeso y obesidad (57). Además del IMC, se están utilizando otros marcadores como la circunferencia de cintura, la circunferencia de cadera, y la ratio cintura/cadera para determinar el grado de adiposidad central y total. Más recientemente, se ha propuesto el perímetro de cuello como un marcador antropométrico sencillo que se asocia significativamente a marcadores convencionales relacionados con la adiposidad total y central tales como tales como el IMC (58) o la circunferencia de cintura, respectivamente (59). Además, el perímetro de cuello también se asocia a factores de riesgo cardiovascular tales como triglicéridos, colesterol total, lipoproteínas de baja densidad (60), glucosa (61) y andrógenos en mujeres pre-menopáusicas con sobrepeso y obesidad (62).

Dentro de las ventajas comparativas que tiene el perímetro de cuello sobre otros marcadores de adiposidad, destaca que es fácil de medir, no cambia en el transcurso del día, no se ve influenciado por la distensión abdominal ocasionada por los alimentos ingeridos, no se altera con la inhalación o exhalación y es práctico, ya que puede medirse fácilmente incluso en invierno cuando las personas utilizan una mayor cantidad de prendas de vestir (63). Esto es especialmente útil sobre todo en aquellas personas que están estigmatizadas por su peso corporal y tienen fobia por pesarse, y en aquellas circunstancias en las que retirar la ropa para medir la circunferencia de cintura no es viable.

Sorprendentemente, los últimos hallazgos han mostrado que el perímetro de cuello sería una medida indirecta de la grasa subcutánea de la parte superior del cuerpo (22) (depósito de grasa ubicado en un compartimento anatómico separado de la grasa subcutánea abdominal), que se asocia con indicadores convencionales de adiposidad total y central y factores de riesgo cardiovascular

independientemente del IMC y del tejido adiposo visceral (22; 23), lo que sugiere que este tipo de grasa sería un depósito patógeno con características únicas.

Con el objetivo de estudiar de manera detallada la relación entre perímetro de cuello con marcadores de adiposidad, se realizó una revisión sistemática. El objetivo de esta revisión fue realizar una búsqueda sistematizada acerca de la validez del perímetro de cuello como marcador de adiposidad en niños y adultos.

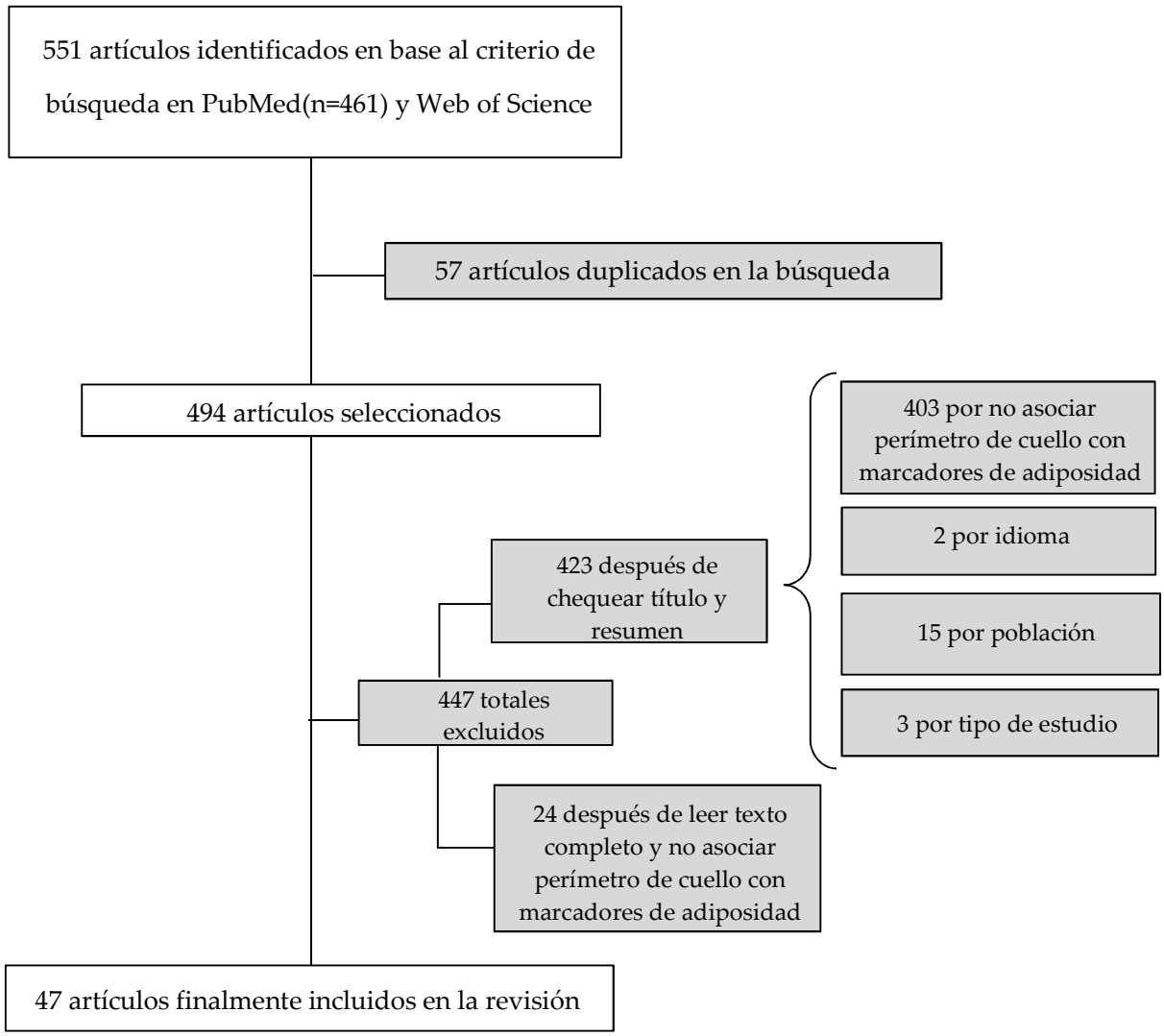
A continuación, la metodología utilizada, junto con los resultados y discusión de los principales hallazgos:

Metodología

Estrategia de búsqueda

Se realizó una búsqueda sistemática de los artículos publicados con anterioridad al 30 de junio del 2017 en PubMed y Web of Science. Se buscaron estudios que analizaran la relación entre el perímetro de cuello y un indicador de adiposidad tanto en niños como en población adulta. Se optó por utilizar términos muy genéricos para intentar identificar todos los estudios que analicen perímetro de cuello y al menos un marcador de adiposidad. Por lo tanto, en PubMed se usaron los términos MeSH (Medical Subject Heading). El criterio de búsqueda utilizado fue: **((“neck circumference” or “neck diameter”) AND ("Body Composition"[Mesh] OR "Anthropometry"[Mesh])**. Se repitió la misma estrategia de búsqueda y combinación de términos en Web of Science, aunque sin usar los términos MeSH, ya que está opción no existe en dicho buscador.

Figura 5. Diagrama de búsqueda de literatura y proceso de selección de artículos.



Criterios de inclusión

Los criterios de inclusión fueron: i) estudios originales; ii) estar escrito en inglés o español; iii) estudio realizado en humanos mayores de 2 años de edad; iv) que incluyan medidas de perímetro de cuello y algún otro marcador de adiposidad (ver abajo), y que éstas sean relacionadas entre sí (analizando su concurrencia o validez); v) estar disponibles como texto completo desde los accesos de los que disponían los autores. Un investigador (MJAT) revisó en detalle si los artículos

cumplían los criterios de inclusión establecidos en dos fases: i) lectura de título y resumen y; ii) lectura de texto completo de los artículos incluidos en la fase anterior.

Marcadores de adiposidad

En esta revisión se consideraron dos niveles de marcadores de adiposidad: (i) aquellos medidos mediante métodos de referencia o indirectos tales como la resonancia magnética nuclear, TAC, DXA, o la pletismografía, y (ii) aquellos medidos con métodos doblemente-indirectos, tales como la bioimpedanciometría (deriva de la medición de agua corporal total), antropometría bicompartimental (comprende mediciones para el cálculo de densidad corporal, las cuales provienen de regresiones lineales en base al método de peso hidrostático), y marcadores como el peso, el IMC, la circunferencia de cintura o la ratio cintura/cadera. Se considera validez de criterio a lo estudiado en aquellos estudios que analizan la asociación entre perímetro de cuello y un marcador de adiposidad medido mediante un método de referencia mientras que se considera un estudio que analiza la validez concurrente cuando el método utilizado para determinar la adiposidad es un método doblemente-indirecto.

Resultados

Se identificaron un total de 551 estudios, de los cuales, el 10% (n=57) estaban duplicados en PubMed y Web of Science. En una primera fase de lectura de título y resumen se eliminaron un total de 423 artículos. Finalmente, tras revisar los textos completos de los 91 artículos restantes para comprobar si los estudios cumplían los criterios de inclusión, se seleccionaron un total de 47 estudios (ver Figura 1).

Las tablas 2 y 3 resumen la totalidad de artículos seleccionados en niños y adultos, respectivamente. De los estudios incluidos en esta revisión, un 38% incluyeron niños o adolescentes (4 estudios en niños, 10 en adolescentes y 4 en

ambos grupos de edad) (Tabla 2), mientras que el 62% (n=29) se realizó en población adulta o adulta mayor (Tabla 3). Los protocolos de medición de perímetro de cuello varían entre estudios. El 51% de los estudios reportan el uso de una cinta métrica plástica no distensible, mientras que el 49% no lo específica. En niños y adolescentes, la medición del perímetro del cuello que predominó fue aquella realizada en la porción más prominente del cuello (23,4%, n = 11), mientras que un 12,8% (n = 6) la realizó en el punto medio o sobre o bajo la prominencia laríngea y un 2,1% (n = 1) no especificó la metodología utilizada. En la población adulta, el 21,3% (n = 10) de los estudios miden el perímetro de cuello en el punto medio del cuello, un 36,2% (n = 17) lo miden sobre o bajo la prominencia laríngea (cartílago cricotiroídeo), mientras que solo el 2,1% (n = 1) de estudios realizó esta medición en la circunferencia mínima y 2,1% (n = 1) no reportó la forma de medición.

Con respecto a los puntos de corte de perímetro de cuello para diagnosticar sobrepeso y obesidad, Ben-Noun y cols (64) fueron los primeros en establecer puntos de corte para identificar sobrepeso (37 y 34 cm en hombres y mujeres respectivamente) y obesidad (39,5 y 36,5 cm en hombres y mujeres adultos respectivamente). Posteriormente, se han propuesto puntos de corte similares (65-67), aunque con ligeras modificaciones en el punto anatómico de medición. En el caso de la población pediátrica, el punto de corte del perímetro de cuello asociado a sobrepeso u obesidad depende de la edad y del grado de maduración sexual, pudiendo fluctuar entre 28 a 38 cm en niños y de 27 a 34,5 en niñas (68-72). Más recientemente, Castro-Piñeiro y col. (73) proponen puntos de corte de perímetro de cuello para niños y niñas de 6 a 18 años asociado a sobrepeso obesidad así como asociado a circunferencia de cintura de riesgo (Tabla 2).

Todos los estudios incluidos en esta revisión mostraron relaciones estadísticamente significativas entre perímetro de cuello y los marcadores de adiposidad total y central, así como con otros marcadores de composición

corporal tales como masa libre de grasa, o índice de masa grasa tanto en niños (Tabla 2) como en adultos (Tabla 3).

Validez de criterio: asociación del perímetro de cuello con marcadores de adiposidad determinados con métodos indirectos o de referencia.

- Niños y adolescentes: No se encontraron estudios que analicen la asociación del perímetro de cuello con un marcador de composición corporal determinado mediante métodos de referencia, por lo que no se puede establecer la validez de criterio del perímetro del cuello en esta población.
- Adultos: un total de cinco estudios mostraron asociación del perímetro de cuello con masa grasa de cuerpo completo determinada mediante TAC en participantes de ambos sexos, de nacionalidad estadounidense (n=3) (74-76), china (n=1) (77) e inglesa (n=1) (78) (Tabla 3). En los 5 estudios, esta técnica permitió estimar la cantidad de tejido adiposo visceral, que también se asoció de forma positiva y significativa con el perímetro de cuello en la totalidad de los casos. Además, tres de los estudios (75-77), encontraron relación directa y significativa entre perímetro de cuello y tejido adiposo subcutáneo y uno de ellos (76), incluye la medición de los compartimientos del tejido adiposo del cuello subdividido en subcutáneo, ubicado entre la piel y fascia cervical profunda, posterior, entre el esternocleidomastoideo, escaleno y trapecio y perivertebral entre los músculos que rodean las cervicales.

  Un total 2 artículos realizaron el análisis de composición corporal mediante DXA, calculando la cantidad de grasa corporal total y abdominal en ambos casos. La población estudiada fue estadounidense (79) y canadiense (80) y los resultados muestran una asociación directa y significativa con el perímetro de cuello (Tabla 3).

  Validez concurrente: asociación del perímetro de cuello con marcadores de adiposidad determinados con métodos doblemente-indirectos

- Niños y adolescentes: Se identificaron tres estudios que analizaron la asociación entre perímetro de cuello y marcadores de adiposidad mediante bioimpedanciometría (81-83). (Tabla 2). La población estudiada (estadounidense, europea y brasileña), incluyó a niños y/o adolescentes de ambos sexos, mayores de 4 años. Es importante destacar, que la raza fue una variable considerada en 1 (81) de los 3 estudios. Finalmente, en este grupo de edad, un 34% (n=16) de los 47 estudios seleccionados en esta revisión, muestran asociación directa y significativa entre perímetro de cuello con IMC, circunferencia de cintura y/o ratio cintura/cadera, independiente del sexo y la edad. Además, Castro-Piñeiro y col. (84) mostró una asociación positiva entre el perímetro del cuello e IMC, circunferencia de cintura, la ratio circunferencia de cintura y altura, porcentaje de masa grasa estimado mediante pliegues cutáneos, e índice de masa grasa estimado también mediante pliegues cutáneos en niños y adolescentes españoles (Tabla 2). Este estudio aporta puntos de corte de perímetro de cuello asociado a IMC y circunferencia de cintura para niños y niñas de 6 a 18 años que oscilan entre 25 y 37 cm.
- Adultos: Se identificaron 7 estudios que mostraban una asociación directa del perímetro de cuello con el porcentaje de masa grasa total, medido con bioimpedanciometría (58; 67; 85-89). El 42% (n=3) de los estudios, realizó el análisis en población japonesa (2 de ellos incluyendo sólo a mujeres postmenopáusicas) (87; 89) y el 58 % restante en búlgara (n=1) (67), coreana (n=1) (88), brasileña (n=1) (85) y puerto riqueña (n=1) (58). En relación a la asociación de perímetro de cuello solo con marcadores convencionales tales como el IMC, circunferencia de cintura y/o ratio cintura/cadera, un 32% (n=15) (59; 61; 64-66; 90-99) de los estudios incluidos en esta revisión, establece relación directa en ambos sexos y todos los grupos de edad.

Tabla 2. Estudios que analizan la asociación entre perímetro de cuello y marcadores de adiposidad en niños.

Autor	Sujetos	Edad (años)	Protocolo medición perímetro de cuello	Método/ indicador de adiposidad	Principales resultados y concl
Hatipoglu et al. 2010(1).	969 turcos: 204 niños y 208 niñas con sobrepeso- obesidad y 271 niños y 284 niñas saludables.	6-18	CMplas, Promcue	IMC y Cc	IMC: Niños-pre púberes: (r=0.70, p<0.001) y púberes (r= Niñas-pre púberes: (r=0.72, p<0.001) y púberes (r= Cc: Niños-pre púberes: (r=0.73, p<0.001) y púberes (r= Niñas-pre púberes: (r=0.77, p<0.001) y púberes (r=
Mazicioglu et al. 2010(2).	5481 niños(as) y adolescentes turcos: 2026 niños y 2519 niñas.	6-18	CMplas, Promcue	IMC	IMC, independiente del rango de edad y sexo (p<
Nafiu et al. 2010(3).	1102 estadounidenses (573 niños y 529 niñas).	6- 18	CMplas, Promcue	IMC, Cc	IMC: Niños (r=0.71, p< 0.001); Niñas (r=0.78, p< 0.0 Cc: Niños (r=0.77, p< 0.001; Niñas (r=0.83, p< 0.001) Al analizar los datos, por rango de edad (6-18 años mantiene (p< 0.001).
Guo et al. 2012(4).	6802 chinos: 3631 niños y 3171 niñas.	5-18	CMplas, Promcue	IMC, Cc	IMC: normopeso (r = 0.68, p < 0.001), sobrepeso (r = 0.29, p < 0.001). Cc: normopeso (r = 0.75, p < 0.001), sobrepeso (r = 0 p < 0.001). Covariables: edad, genero, IMC o Cc según corresp correlación con mantención de la significación. Sol es correlacionado al ser ajustado (r=-0.004, p=0.932)

Lou et al. 2012(5).	2847 chinos: 1475 niños y 1372 niñas.	7-12	CMplas, SCtir	IMC y Cc	IMC: Niños (r=0.80, p< 0.001); Niñas (r=0.73, p< 0.0 Cc: Niños (r=0.80, p< 0.001); Niñas (r=0.73, p< 0.001 Al analizar los datos, por rango de edad (7-12 años mantiene (p< 0.001).
Kurtoglu et al. 2012(6).	461 turcos: 199 niños y 262 niñas.	5 y 18	CMplas, Promcue	IMC, Cc	IMC: Niños-pre púberes: (r=0.75, p<0.001) y púberes (r= Niñas-pre púberes: (r=0.78, p<0.001) y púberes (r= Cc: Niños-pre púberes: (r=0.82, p<0.001) y púberes (r= Niñas-pre púberes: (r=0.85, p<0.001) y púberes (r=
Phan et al. 2012(7).	152 obesos estadounidenses: 76 niños y 76 niñas.	13	Promcue	Porcentaje de grasa (BIA)	Porcentaje de grasa corporal total (medido en cada raza; existiendo mayor relación de esta medición a no blancos (p<0.05).
Bammann et al. 2013(8).	78 niños de 4 países de la Unión Europea: 35 niños y 43 niñas.	4-10	CMmet, SCtir	Porcentaje de gr(9)asa (BIA)	Masa grasa (R^2=0.48, p<0.001). Covariables: edad y sexo.
Nafiu et al. 2013(10).	1058 estadounidenses: 561 niños y 497 niñas.	6-18	CMplas, Promcue	IMC, Cc	IMC: Niños (r=0.72, p<0.001); Niñas (r=0.71, p<0.00 Cc: Niños (r=0.78, p<0.001); Niñas (r=0.83, p<0.001)
Sacco et al. 2013(11).	98 brasileros: 43 niños y 55 niñas.	5	NR	Peso corporal, IMC y Cc	A un mayor peso de nacimiento (>+0.67 DS, p=0.01 Rápida ganancia de peso hasta los 2 (>+1 DS, p=0.0 Obesidad materna (>+2 DS, p<0.001).
Coutinho et al. 2014(9).	2794 brasileros: 1394 niños y 1400 niñas.	6-18	Promcue	IMC, Cc y porcentaje grasa corporal (BIA)	El perímetro de cuello fue correlacionado en ambos IMC, Cc y porcentaje de grasa corporal total (p<0.0

Da Silva et al. 2014(12).	388 brasileros: 169 niños y 219 niñas.	10 – 19	PMcue	IMC y Cc	IMC: Niños-pre púberes: (r=0.57, p<0.001) y púberes (r= Niñas-pre púberes: (r=0.39, p<0.01) y púberes (r= 0. Cc: Niños-pre púberes: (r=0.82, p<0.001) y púberes (r= Niñas-pre púberes: (r=0.51, p<0.001) y púberes (r= Covariables: porcentaje de grasa corporal.
Katz et al. 2014(13).	1913 canadienses: 977 niños y 936 niñas.	6-17	CMplas, Promcue	IMC y Cc	IMC (al categorizar la muestra como estado nutrici Cc en niños y niñas, p-value(beta) < 0.0001, para to Covariables: estado nutricional (peso normal, sobr
Ferreti et al. 2015(14).	1668 brasileros: 794 niños y 916 niñas.	10-17	CMplas, PMcue	IMC, Cc, porcentaje de grasa (antropometría bicompartimental)	IMC (OR crudo: 3.83, p<0.001; OR ajustado: 1.21, p Cc (OR crudo: 1.13, p<0.001; OR ajustado: 1.02, p=0 Porcentaje de grasa corporal total (OR crudo: 1.03, p<0.001). El punto de corte de perímetro de cuello para obes IMC (OR crudo: 23.5, p<0.001) Cc (OR crudo: 1.10, p<0.001) Porcentaje de grasa corporal total (OR crudo: 1.05, p=0.048). Covariables: no reportadas.
Formisano et al. 2016(15).	15673 europeos: 7962 niños y 7711 niñas.	3-10	CMplas, Promcue	Cc	z-core de Cc: Niños (r=0.31, p<0.001); Niñas (r=0.35
Kelishadi et al. 2016(16).	23.043 iraníes: 13549 niños y 9494 niñas.	6-18	CMplas, BCtir	IMC, Cc, RC/cad, Cc/talla, Ccad	IMC (r=0.38, p<0.001). Cc (r=0.47, p<0.001). RC/cad, (r=0.023, p<0.001). Cc/talla (r=0.18, p<0.001). Ccad (r=0.47, p<0.001). Al analizar los datos, por rango de edad (6-18 año mantiene (p<0.001).

					Covariables: edad, sexo, área geográfica de reside sobrepeso y obesidad abdominal); modelo no difie
Castro-Piñeiro J. et al. (17)	2198 españoles: 1277 niños y 921 niñas.	6-18	CMplas BCtir,	IMC, Cc, Cc/talla, porcentaje de grasa corporal total (antropometría) .	IMC: Niños (r=0.75, p<0.001); Niñas (r=0.79, p<0.0 Cc: Niños (r=0.86, p<0.001); Niñas (r=0.85, p<0.001 Cc/talla: Niños (r=0.61, p<0.001); Niñas (r=0.62, p< Porcentaje de grasa corporal total: Niños (r=0.55, p Índice de masa grasa total: Niños (r=0.49, p<0.001 (r=0.47, p<0.001).
Kondolot et al. 2017(18).	1766 turcos: 874 niños y 892 niñas.	2-6	CMplas, Promcue	IMC clasificado en percentiles	El perímetro de cuello se incrementa con la edad, s niños(as) con obesidad, definida según IMC ≥p95.

Protocolo de medición: CMplas: cinta métrica plástica; CMmet: cinta métrica metálico; PMcue: punto medio del cuello: CMcue: ci
Promcue: porción más prominente del cuello; SCtir: sobre cartílago tiroideo; BCtir: bajo cartílago tiroideo: NR: no reporta. M
IMC: índice masa corporal; Cc: Circunferencia de cintura; Ccad: circunferencia de cadera; RC/cad: índice cintura/cadera; TAC: to
BIA: bioimpedanciometría; DXA: absorciometría dual de rayos X.

Tabla 3. Estudios que analizan la asociación entre perímetro de cuello y marcadores de adiposidad en adultos.

Autor	Participantes	Edad (años)	Protocolo medición perímetro de cuello	Método/Indicador de adiposidad	Principales resultados y
Yang et al. 2009(19).	18 pacientes obesos no diabéticos ingleses.	22- 66	CMplas, PMcue	TAC (estimación de tejido adiposo visceral en L4).	Tejido adiposo visceral ($r^2 = 0.67$, p=
Preis et al. 2010(20).	3307 estadounidenses: 1718 hombres y 1589 mujeres.	51	CMplas, BCtir	IMC, Cc, TAC	IMC (hombres r = 0.79, p=<0.0001; m Cc (hombres r = 0.75, p=<0.0001; mu Tejido adiposo visceral (hombres r = 0.74, p=<0.0001) Covariables: edad
Li HX et al. 2014 (21).	Datos recolectados 177 chinos: 87 hombres y 90 mujeres.	35 - 75	CMplas, SCtir	TAC, IMC, Cc, RC/cad.	Tejido adiposo visceral: hombres (r= 0.25, p>0.05). Tejido adiposo subcutáneo hombres (r=0.41; p>0.001).
Rosenquist et al. 2014(22)	91 adultos de la cohorte Framingham:46 hombres y 45 mujeres	58.5	BCtir	IMC, Cc, TAC	IMC (r=0.73, p<0.001). Cc (r=0.65, p<0.001). Tejido adiposo visceral (r=0.71, p<0.0 Tejido adiposo subcutáneo (r=0.56, p Covariables: edad y sexo

Torriani et al.2014(23).	303 estadounidenses: 152 hombres y 151 mujeres.	55 ±17	NR	TAC, IMC y Cc.	IMC (hombres r=0.70, p<0.001; mujer Cc (hombres r=0.71, p<0.001; mujeres Tejido adiposo visceral (hombres r=0 p<0.001) Tejido adiposo subcutáneo (hombres r=0.63, p<0.001). Covariables: edad, estado de enferm
Cizza et al. 2014(24).	120 estadounideneses: 28 hombres y 92 mujeres.	18-50	CMcue	Porcentaje de grasa corporal (DXA)	Porcentaje de grasa abdominal total, porcentaje de grasa subcutánea (r=0.4
Ravensberge n et al. 2014(25).	27 canadienses	40	PMcue	Porcentaje grasa corporal total, grasa abdominal (DXA).	Gramos de grasa corporal total (r=0.6 Gramos de grasa abdominal (r=0.63,
Ben-Noun et al. 2001(26).	735 israelís: 460 hombres y 519 mujeres.	35 -65	CMplas, PMcue	IMC, Cc, Ccad, RC/cad	IMC (hombres r=0.83, mujeres r=0.71 Peso (hombres r=0.70, mujeres r=0.81 Cc (hombres r=0.86, mujeres r=0.85, p Ccad (hombres r=0.62, mujeres r=0.5 RC/cad (hombres r=0.66, mujeres r=0
Ben-Noun et al. 2003(27).	561 israelís: 231 hombres y 330 mujeres.	18 o más	CMplas, PMcue	IMC, Cc, RC/cad	IMC (hombres r = 0.71; mujeres r = 0. Cc (hombres r = 0.75; mujeres r = 0.7 RC/cad (hombres r = 0.56; mujeres r

Feet et al. 2005(28).	55 mujeres brasileñas.	36±10	CMplas, PMcue	IMC, Cc, RC/cad, porcentaje de grasa (antropometría, BIA)	El perímetro de cintura se asoció pre (ejercicio y dieta) con: IMC (pre r=0.71- post r=0.68, p=0.000 Cc (pre r=0.67, p=0.0001; post r=0.62, RC/cad (pre r=0.48, p=0.0004; post r= Porcentaje de grasa según antropom post r=0.60, p=0.001) Porcentaje de grasa según bioimpeda post r=0.74, p=0.0002).
Ben-Noun et al. 2006 (29).	364 israelís: 155 hombres y 209 mujeres.	18 o más	CMplas, PMcue	IMC, Cc, RC/cad	Los cambios de perímetro de cuello s IMC (hombres r=0.79, p<0.001; mujer Cc (hombres r=0.75, p<0.001; mujeres RC/cad (hombres r=0.47, p<0.001; mu Covariables: edad
Davidson et al. 2008(30).	598 estadounidenses: 424 hombres y 174 mujeres.	48	PMcue	IMC, Cc, RC/cad	IMC (hombres r = 0.66, p=<0.001; muj Cc (hombres r = 0.61, p=<0.001; mujer RC/cad (hombres r = 0.35, p=<0.001; r p=<0.001)
Onat et al. 2009(31).	1912 turcos: 934 hombres y 978 mujeres.	55.1 +/- 12	PMcue	IMC, Cc, ratio cintura/cadera	IMC (hombres r = 0.69, p=<0.001; mu p=<0.001) Cc (hombres r = 0.70, p=<0.001; muje Ratio cintura/cadera (hombres r = 0.4 mujeres r = 0.22, p=<0.001).
Kawaguchi et al. 2010(32).	219 japoneses: 170 hombres y 49 mujeres.	52.8 ± 15.0	PMcue	Porcentaje de grasa corporal (BIA)	Porcentaje de grasa visceral (*r* = 0.731 Al realizar el análisis del perímetro c relación se incrementa (r = 0.819, p <

Yang et al. 2010(33).	3182 diabéticos chinos: 1294 hombres y 1888 mujeres.	20-80	SCtir	IMC, Cc	IMC (hombres r = 0.41, p=<0.0001; m p=<0.0001) Cc (hombres r = 0.47, p=<0.0001; muj p=<0.0001)
Hingorjo et al. 2012(34).	150 pakistaníes: 41 hombres y 109 mujeres.	18-20	CMplas, SCtir	IMC, Cc,Cdad, RC/cad	IMC (hombres r = 0.86, p=<0.001; mu p=<0.001) Cc (hombres r = 0.85, p=<0.001; mujer Ccad (hombres r = 0.82, p=<0.001; mu p=<0.001) RC /cad(hombres r = 0.69, p=<0.001; r p=<0.05).
Akin et al. 2013(35)	92 hombres turcos.	40 – 60	CMplas, SCtir	IMC, Cc	IMC (r = 0.7, p < 0.001). Cc (r = 0.6, p < 0.001).
Stabe et al. 2013(36).	1053 brasileros: 301 hombres y 752 mujeres.	18-60	BCtir	IMC, Cc, RC/cad	IMC: hombres (r = 0.67, p=<0.001); m Cc: hombres (r = 0.71, p=<0.001); muj RC /cad (hombres r = 0.33, p=<0.001; Covariables: edad
Aoi et al. 2014(37).	64 mujeres sanas postmenopáusicas japonesas	63.6 ± 7.1	BCtir	IMC, Cc, porcentaje de grasa (BIA).	IMC (r=0.74, p =0.001). Cc (r=0.72, p =0.001). Porcentaje de grasa (r=0.71, p=0.001).
Saka et al. 2014(38).	411 turcos: 174 hombres y 237 mujeres.	20 -60	CMplas, BCtir	IMC, peso corporal, Cc, Ccad y RC/cad	Peso corporal (hombres; r=0.57, p<0. p<0.001). IMC (hombres r=0.58, p<0.0001mujer Cc (hombres r=0.59, p<0.0001, mujere Ccad(hombres r=0.56, p<0.0001 muje

					RC/cad (hombres r=0.27, p<0.0001 m
Yan et al. 2014(39)	2092 chinos: 971 hombres y 1121 mujeres.	65	SCtir	IMC, Cc	IMC (hombres r=0.70, p<0.01, mujere Cc (hombres r=0.73, p<0.01 mujeres r
Cho et al. 2015(40).	3521 datos de no diabéticos coreanos: 1784 hombres y 1737 mujeres.	42-71	BCtir	IMC, Cc, porcentaje de grasa (BIA).	IMC (hombres r=0.80, p<0.001; mujer Cc (hombres r=0.74, p<0.001; mujeres Porcentaje de grasa total (hombres r= p<0.001).
Özkaya et al 2016(41).	1157 turcos: 319 hombres y 838 mujeres.	18- 24	CMplas, PMcue	IMC, Cc, Ccad, RC/cad	IMC (hombres r=0.68, p<0.01, mujere Cc (hombres r=0.68, p<0.01, mujeres Ccad (hombres r=0.64, p<0.01, mujer RC/cad (hombres r=0.64, p<0.01, muj
Aoi et al. 2016(42).	63 mujeres sanas postmenopáusicas japonesas	NR	BCtir	IMC, Cc, porcentaje de grasa (BIA).	IMC (r=0.74, p =0.001) Cc (r=0.73, p =0.001) Porcentaje de grasa (r=0.74, p=0.001) Tras 3 años de seguimiento, los camb también fueron asociados a IMC (r = =0.045) y porcentaje de grasa (r = 0.2, Covariables: edad
Assyov et al. 2016(43).	255 búlgaros:102 hombres y 153 mujeres	49 ± 12	BCtir	IMC, porcentaje de grasa (BIA).	IMC (hombres r=0.29, p<0.05; mujere Porcentaje de grasa corporal (hombr r=0.46, p<0.01). Covariables: edad

Baena et al. 2016 (44).	15.105 brasileros: 3810 hombres y 4916 mujeres.	35-74	SCtir	IMC, Cc	IMC (hombres r=0.72, p<0.001; mujer Cc (hombres r=0.72, p<0.001; mujeres Covariables: edad
Coelho et al. 2016 (45).	435 adultos mayores brasileros: 64 hombres y 371 mujeres.	>60	SCtir	IMC, Cc, RC/cad	IMC, Cc y RC/cad: *Valores de r y su en el artículo.
Joshipura et al. 2016(46).	Datos de 1206 adultos no diabéticos puertorriqueños	40-65	CMplas, BCtir	IMC, Cc y porcentaje de grasa corporal (BIA)	IMC (r=0.66, p<0.001) Cc (r=0.64, p<0.001) Porcentaje de grasa corporal (r=0.45, Covariables: edad, sexo, tabaquismo
Limpawattana et al. 2016(47).	587 tailandeses: 201 hombres y 386 mujeres.	≥50	SCtir	Cc	Cc (hombres: r=0.7, p<.001; mujeres:

Protocolo de medición: CMplas: cinta métrica plástica; CMmet: cinta métrica metálico; PMcue: punto medio del cuello: CMcue: ci
Promcue: porción más prominente del cuello; SCtir: sobre cartílago tiroideo; BCtir: bajo cartílago tiroideo: NR: no reporta. M
IMC: índice masa corporal; Cc: Circunferencia de cintura; Ccad: circunferencia de cadera; RC/cad: índice cintura/cadera; TAC: to
BIA: bioimpedanciometría; DXA: absorciometría dual de rayos X.

Discusión

Los resultados de la presente revisión sistemática muestran que el perímetro del cuello se asocia de forma directa con marcadores de adiposidad medidos mediante métodos de referencia tales como el TAC o el DXA en adultos, mientras que no se encontraron estudios en niños. Además, se observó que el perímetro del cuello se asociaba consistentemente en todos los estudios con marcadores de adiposidad total y central tales como el IMC, perímetro de cintura y ratio cintura/cadera tanto en población adulta como en niños. El perímetro de cuello es un método sencillo, inocuo, rápido, de bajo coste, no influenciable por el ayuno-saciedad, vestimenta, temperatura ambiente o limitaciones socioculturales. Además, dada la existencia de puntos de corte para el diagnóstico de sobrepeso y obesidad, apuntan a una gran utilidad de este marcador tanto en investigación como en clínica. Sin embargo, el reducido número de estudios de validación frente a métodos de referencia "gold standard" en adultos, y la ausencia de estudios con métodos de referencia "gold standard" en niños y adolescentes ponen de manifiesto la necesidad de realizar nuevos estudios que analicen la validez de criterio del perímetro de cuello como indicador de adiposidad total, central y visceral.

En el caso de la población infantil, no se encontraron estudios que analicen la relación entre el perímetro de cuello y otros indicadores de adiposidad en base a métodos considerados de referencia, sin embargo, todos los resultados obtenidos en base a relación esta medición antropométrica con marcadores convencionales, son positivos y estadísticamente significativos. En relación a los estudios que utilizaron bioimpedancia en población infantil (81-83), el perímetro de cuello fue correlacionado con el porcentaje de masa grasa en niños estadounidenses, europeos y brasileños. Cabe destacar que un hallazgo novedoso, es encontrado en 76 adolescentes obesos de 13 años (81), en los cuales se observó que en etnias no caucásicas, parece que la relación entre perímetro de cuello y porcentaje de

masa grasa es más fuerte ($p<0.05$), resultado que hasta la fecha no ha sido reportado en población adulta.

El crecimiento durante la etapa escolar podría estar influenciando directamente el punto anatómico de medición más utilizado en la mayoría de los estudios que trabajaron con este grupo de edad. En el 56% de los estudios identificados midieron el cuello en la porción más prominente. Por otro lado, es posible que el perímetro de cuello pueda ser una medición muy útil desde los primeros años de vida. De hecho, un estudio en una cohorte de 98 niños brasileros, concluyó que un mayor perímetro de cuello a los 5 años de edad, estaría directamente vinculado a una mayor ganancia de peso hasta los 2 años de edad (102). Estos resultados indican que el perímetro del cuello se puede utilizar como un marcador predictor de sobrepeso y obesidad. Aunque hacen faltan más estudios para confirmar esta hipótesis.

Todos los estudios que analizaron la validez de criterio en adultos y adultos mayores, reportaron asociación positiva entre perímetro de cuello y adiposidad total, abdominal, visceral y/o subcutánea. El primer estudio que utilizó la técnica TAC para estudiar la asociación entre perímetro de cuello y masa grasa visceral fue realizado por Yang y col. (78) en 18 obesos no diabéticos ingleses entre 22 y 66 años de edad. Los estudios realizados por Preis y col. (74), Li y col. (77), Rosenquist y col. (75) y Torriani y col. (76), refuerzan los mismos resultados. Torriani y col. (76) además examinaron la relación entre el perímetro con masa grasa posterior, subcutánea y perivertebral del cuello, describiendo que el perímetro de cuello aumentaría en un 30% (mujeres) y 24% (hombres) al comparar los grupos normopeso vs obesos, y que los compartimientos adiposos del cuello se expandirían de diferente manera al incrementarse el IMC. Además, mostraron que el tejido adiposo perivertebral tendría una menor capacidad de almacenamiento de masa grasa, repercutiendo en los depósitos de los compartimientos posterior y subcutáneo, los cuales estarían relacionados con

factores de riesgo cardiovascular en el grupo de mujeres (p<0,001). Además, los dos estudios que realizaron el análisis de composición corporal mediante DXA, también encontraron asociación positiva con perímetro de cuello. Cizza y col. (79) en una muestra de 120 estadounidenses entre 18 y 50 años, mostraron que el perímetro de cuello se relaciona con el porcentaje de grasa abdominal total, visceral y subcutánea. Resultados similares se describen en el estudio de Ravensbergen y col. (80), el mismo año, en 27 participantes canadienses de 40 años, en los cuales se encontró correlación entre perímetro de cuello con porcentaje de grasa corporal total y abdominal (r=0,6; p= 0,003 y r=0,63, p= 0,002, respectivamente). En relación a los estudios que utilizan métodos de composición corporal indirectos, estos se caracterizaron por considerar diversidad de raza, edad e incluir población saludable. La excepción a la regla con respecto al tipo de condición fisiopatológica de población investigada, fue publicada el año 2010 por Yang y col. (65) en 3182 diabéticos chinos entre 20-80 años, con resultados similares de relación de perímetro de cuello con IMC y circunferencia de cintura a los hallazgos anteriormente mencionados.

Por último, la ausencia de estudios que ajusten el análisis por posibles variables confusoras tales como el sexo, la edad y porcentaje de adiposidad total, junto con el hecho de que da Silva y col. (103) en 388 brasileros entre 10 y 19 años en un reciente estudio, encontraron que la asociación entre perímetro de cuello e IMC podría ser independiente de masa magra, sugieren que las futuras líneas de investigación deberían analizar la relación de este indicador también con masa magra, en diferentes edades, etnias, condiciones fisiológicas y/o patológicas.

Conclusiones

El perímetro de cuello se asocia a marcadores indirectos de masa grasa total y central indirectos en niños y adolescentes. En adultos, no hay duda de que perímetro de cuello es un marcador válido para medir adiposidad total y central. Se requieren más estudios con métodos que analicen la asociación entre el

perímetro del cuello y adiposidad analizada mediante métodos de referencia en niños y adolescentes. También es necesario analizar si el perímetro del cuello se asocia a otros parámetros de composición corporal tales como la masa magra tanto en niños y adolescentes como en adultos.

iv) Tejido adiposo del cuello

Más recientemente, Lee y cols. (108) mostraron en 2306 participantes del estudio "Framingham" que una mayor cantidad de grasa subcutánea en la parte superior del cuerpo está relacionada con factores de riesgo cardiometabólico, independientemente del IMC, perímetro de cuello y tejido adiposo visceral, lo que sugiere que otros factores, como depósitos ectópicos, podrían estar explicando el riesgo adicional perdido por la grasa acumulada a nivel central (109).

Curiosamente, el tejido adiposo del cuello (TA cuello), cuantificado por tomografía computarizada a nivel de C5, ha sido previamente clasificado en tres compartimentos (ver figura 6) (109):

i) *TA cuello Subcutáneo / superficial:* tejido adiposo del cuello que se encuentra entre la piel y la fascia cervical profunda.

ii) *TA cuello intermuscular o posterior:* ubicado entre el músculo esternocleidomastoideo, escaleno y trapecio, separado del compartimento superficial por la fascia cervical profunda.

iii) *TA cuello perivertebral:* tejido adiposo del cuello ubicado alrededor de la vértebra C5.

Figure 6. Anatomía y funcionalidad del tejido adiposo del cuello.

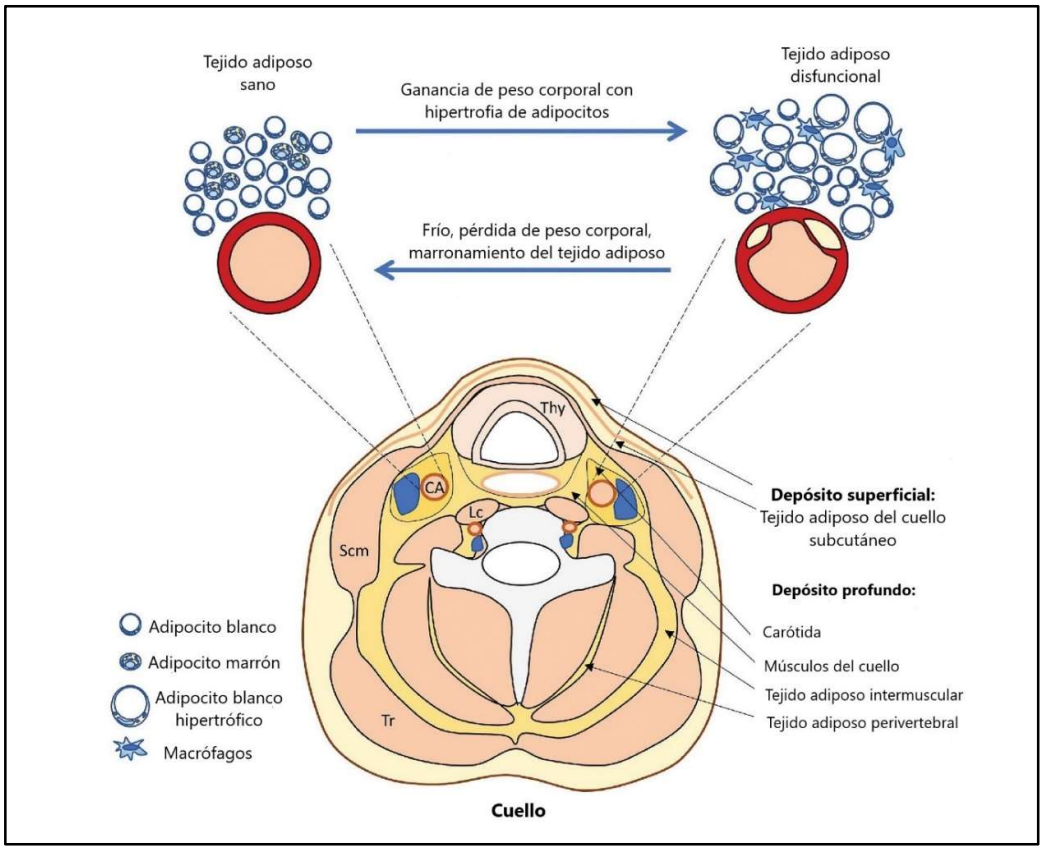


Figura extraída de Pandzic Jaksic y cols (2016) y adaptada por Arias MJ.

En este contexto, Torriani y cols. (110) han mostrado que el TA cuello es mayor cuanto mayor es el IMC, siguiendo diferentes patrones de acumulación según el sexo, y que un aumento de ciertos compartimientos del TA cuello (principalmente profundos) se correlacionaría de manera diferente con los factores de CMR. Sin embargo, los resultados han sido obtenidos de pacientes que habían consultado previamente por ciertos tumores malignos / benignos. Del mismo modo, Rosenquist y cols.(111) en 92 participantes mostraron que la grasa subcutánea del cuello se asocia positivamente con factores RCM (89), sugiriendo la importancia de incrementar estudios de manera aislada de este depósito, debido a que el perímetro cuello (más ampliamente estudiado) solo sería una medida representativa de la grasa subcutánea del tronco superior. Más

recientemente, Tal y cols. (112) han concluido que un mayor volumen del TA cuello en relación a la altura (índice tejido adiposo cuello/ altura) está directamente asociado con mortalidad a largo plazo, independientemente de la edad, el sexo y el estado de diabetes mellitus tipo 2.

Por lo tanto, una mayor cantidad de estudios que permitan determinar el riesgo del TA cuello como un depósito grasa único son de gran interés clínico. Adicionalmente, el estudio sobre TA cuello en personas jóvenes y sin enfermedades crónicas, donde la prevalencia RCM es muy baja o aún puede ser modulada (28, 29), es de gran relevancia.

En conclusión, el TA cuello es un depósito aún poco estudiado. Conocer el mecanismo fisiopatológico subyacente a la acumulación de grasa en el cuello y su vinculación con los diferentes factores de RCM es fundamental para identificar nuevas alternativas y futuras líneas para el diagnóstico y tratamiento de la obesidad.

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03

AIMS

Overall aim
Specific aims

AIMS

Overall aim

To determine the association of neck adipose tissue and circumference with cardiometabolic risk, inflammatory factors, sedentary time, physical activity and to examine the dose-response effect of a concurrent exercise 6-month intervention on the neck measurements in sedentary adults.

Specific aims

1. Specific aim I: To examine the association of neck circumference with indicators of anthropometry and body composition, including total and central body fat as well as lean body mass measured by dual energy x-ray absorptiometry in young Spanish adults (**study 1**).

2. Specific aim II: To examine the association of neck circumference and other simple measures such as waist circumference, body mass index, fat mass and fat mass index with thigh intermuscular adipose tissue and visceral adipose tissue, measured with computed tomography in overweight/obese women (**study 2**).

3. Specific aim III: To examine the relationship of compartmental and total neck adipose tissue with overall and central adiposity, and cardiometabolic risk and inflammatory markers in young sedentary adults (**study 3**).

4. Specific aim IV: To examine: i) whether the time spent in sedentary behaviour and physical activity are related to compartmental and total neck adipose tissue volume in a cohort of young healthy adults; and ii) whether there are differences in the compartmental and total neck adipose tissue

volume across groups of participants who meet and do not meet the international physical activity recommendations (**study 4**).

5. Specific aim V: To examine the dose-response effect of a concurrent exercise intervention of 6 months on the neck adipose tissue and circumference of young healthy adults (**study 5**).

04

METHODOLOGY OVERVIEW OF THE STUDIES INCLUDED

Cross-sectional original studies
Randomized controlled trial

METHODOLOGY OVERVIEW OF THE STUDIES INCLUDED

The methodology of this Doctoral thesis has been structured in cross-sectional original studies (n=4) and 1 randomized controlled trial. The methodological overview has been explained according to this criterion in the tables 1 (studies 1, 2, 3 and 4) and 2 (study 5 about exercise intervention program).

Table 1. Methodology studies 1, 2, 3 and 4.

Study	Tittle	Design	Project	Participants; age	Independent variables (instruments)	Unit	Dependent varia (instruments)
1	Association of neck circumference with anthropometric indicators and body composition measured by DXA in young Spanish adults.	CSS.	ACTIBATE study (1).	119 healthy and sedentary young adults (82 women) of 18 to 25 years old.	• NC (inextensible metallic tape: imt).	cm.	• BMI (bascule/ stadi • TMI (bascule/ stadi • WC (imt) • W/hip • W/height • FMI (DXA) • VAT (DXA) • LMI (DXA)
2	Neck circumference is associated with adipose tissue content in thigh skeletal muscle in overweight and obese premenopausal women	CSS.	Data base of Human Movement, Technical University of Lisbon (2011) (2).	142 premenopausal overweight and obese Caucasian women.	• NC (imt). • WC (imt). • BMI (bascule/ stadiometer). • FM (DXA). • FMI (DXA).	cm. cm. kg/m². kg. kg/m².	• Intermuscular adipo in the tight (CT) • VAT (DXA)

Study	Tittle	Design	Project	Participants; age	Independent variables (instruments)	Unit	Dependent variables (instruments)
3	Neck adipose tissue accumulation is associated with higher overall and central adiposity, cardiometabolic risk, and pro-inflammatory profile in young adults.	CSS.	ACTIBATE study.	139 healthy and sedentary young adults (94 women) of 18 to 25 years old.	• Subcutaneous NAT (CT). • Intermuscular NAT (CT). • Perivertebral NAT (CT). • Total NAT (CT). • NC (imt).	Vol (cm^3). cm.	**Cardiometabolic profile:** • Glucose • Insulin • HOMA • TC • LDL-C • HDL-C • TC/HDL-C • LDL-C/HDL-C • Triglycerides • SBP • DBP (mmHg) • Muscular strength/weigh • CRF/weight • CMR-score (IDF) **Inflammatory profile:** • C-reactive protein • IL-2 • IL-4 • IL-6 • IL-7 • IL-8 • IL-10 • IL-17a • IFNɣ

Study	Tittle	Design	Project	Participants; age	Independent variables (instruments)	Unit	Dependent variables (instruments)
4	Objectively measured sedentary time and physical activity are associated with neck adipose tissue volume in young sedentary adults.	CSS.	ACTIBATE study.	134 healthy and sedentary young adults (92 women) of 18 to 25 years old.	• LPA (Acc). • MPA (Acc). • VPA (Acc). • MVPA (Acc). • MVPA10min (Acc).	min/day.	• Subcutaneous NAT (CT). • Intermuscular NAT (CT). • Perivertebral NAT (CT). • Total NAT (CT). • NC (imt).

ACTIBATE: Activating brown adipose tissue through exercise; Acc: accelerometry; BMI: Body mass index; CSS: Cross-Sectional Study; CT: C
Fat mass index; IDF: International Diabetes Federation; LMI: Lean body mass index; LPA: light physical activity, MPA: moderate physica
vigorous physical activity, MVPA10min: moderate-vigorous physical activity in bouts of 10 minutes, NAT: neck adipose tissue, NC: Neck circ
mass index; VAT: Visceral adipose tissue; VPA: vigorous physical activity; WC: Waist circumference; W/hip: Waist to hip ratio; W/height: Wa

Table 2. Methodology study 5 about exercise intervention program.

Study	Tittle	Design	Project	Participants; age	Independent variables (instruments)	Unit	Covariables
5	Dose-response effect of a concurrent exercise intervention on Neck adipose tissue of young adults: a randomized controlled trial.	Randomized controlled trial.	ACTIBATE study.	56 healthy and sedentary young adults (38 women) of 18 to 25 years old. Group of exercise with strict filter: • Control: 23 • Intermedium:16 • Vigorous:16	• Δ Subcutaneous NAT (CT). • Δ Intermuscular NAT (CT). • Δ Perivertebral NAT (CT). • Δ Total NAT (CT). • Δ NC (imt).	Vol (cm^3). cm.	• Baseline • Sex

ACTIBATE: Activating brown adipose tissue through exercise; CT: Computed tomography; NAT: neck adipose tissue, NC: Neck circumferen

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05

RESULTS & DISCUSSION

Study 1
Study 2
Study 3
Study 4
Study 5

Study 1

Association of neck circumference with anthropometric indicators and body composition measured by DXA in young Spanish adults

ABSTRACT

Background: Due to a clinical and public health interest of neck circumference (NC), a better understanding of this simple anthropometric measurement, as a valid marker of body composition is necessary.

Materials and Methods: A total of 119 young healthy adults participated in this study. NC was measured over the thyroid cartilage and perpendicular to the longitudinal axis of the neck. Body weight, height, waist circumference (WC), and hip circumference were measured. A Dual X-ray absorptiometry (DXA) scan was used to determine fat mass, lean mass, and visceral adipose tissue (VAT). Additionally, body mass index (BMI) and triponderal mass index (TMI), the waist to hip and waist to height ratios, and the fat mass and lean mass indexes (FMI and LMI, respectively) were calculated.

Results: NC was positively associated in women (W) and men (M), with BMI 5432a (rW = 0.70 and rM = 0.84, respectively), TMI (rW = 0.63 and rM = 0.80, respectively), WC (rW = 0.75 and rM = 0.86, respectively), VAT (rW = 0.74 and rM = 0.82, respectively), Waist/hip (rW = 0.51 and rM = 0.67, respectively), Waist/height (rW = 0.68 and rM = 0.83, respectively) and FMI (rW = 0.61 and rM = 0.81, respectively). The association between NC and indicators of body composition was however weaker than that observed by BMI, TMI, WC and Waist/height in both women and men. It is of note that in women, NC was associated with FMI, VAT and LMI independently of BMI. In men, adding NC to anthropometric variables did not improve the prediction of body composition, while slight improvements were observed in women. *Conclusions:* Taken together, the present study provides no indication for NC as a useful proxy of body composition parameters in young adults, yet future studies should explore its usefulness as a measure to use in combination with BMI, especially in women.

Keywords: body fat distribution; cardiovascular risk; neck adipose tissue; obesity; upper body fatness.

INTRODUCTION

Data from the European Health Interview Survey (Eurostat) indicates that more than half of the European population is overweight or obese [1]. Obesity virtually affects all ages and socioeconomic groups, and threatens to overwhelm both developed and developing countries [2]. Furthermore, obesity increases the risk of cardiovascular diseases and premature death [3].

Body weight and height are used to calculate body mass index (BMI) with the aim to classify individuals as underweight, normal weight, overweight, or as having obesity [4]. Nevertheless, BMI does not reflect body fat distribution [5,6]. Both waist circumference and waist-hip circumference ratio are indicators of body fat distribution, and they are strongly associated with cardiovascular disease [7,8]. However, these measures can be affected by the postprandial abdominal distension and breathing movement. In addition, people with or without obesity can have the same waist–hip ratio, making these measurements inappropriate to evaluate obesity [9].

Neck circumference has been proposed as an indicator of upper body fatness [10,11], as it has been associated with overweight and obesity phenotypes [12–15] as well as with cardiovascular disease risk factors [16–20]. Neck circumference is considered a practical measurement because, unlike other methods, it is easy to measure, it does not vary during the course of the day, it does not change with food intake or abdominal distension, it is not altered by inhalation or exhalation, and it can be measured without having to remove clothing [21,22]. The validity of neck circumference against reference methods such as computed axial tomography (TAC) and/or dual X-ray absorptiometry (DXA) has been studied in American [11,12,23,24], Canadian [25], Chinese [26], and English [27] individuals of both sexes, yet the results are limited to the association of neck circumference with total and abdominal body fat as well as with subcutaneous and visceral fat. However, whether neck circumference is

associated with other parameters of body composition, such as lean mass, remains unknown.

Due to the clinical and public health interest about the utility of neck circumference, it is necessary to know if this measure is a valid marker of body composition. In addition, for a better understanding of the utility of neck circumference in the assessment of several chronic diseases in a healthy population, firstly it is necessary validate this anthropometric measure with body composition.

The aim of this study was to examine the association of neck circumference with indicators of anthropometry and body composition, including total and central body fat as well as lean body mass measured by DXA in young Spanish adults.

MATERIALS AND METHODS

Participants

This cross-sectional study included a sample of 119 participants (82 women) aged 18 to 25 years old. The participants were enrolled in the ACTIBATE study (Clinical Trial Registration: NCT02365129 (ClinicalTrials.gov) [28], and were recruited through advertisements in electronic media and leaflets. All assessments were performed in Granada (south of Spain), during the months of October, November, and December 2016. The inclusion criteria were being healthy, not smoking or taking any medication, being sedentary (the participants reported to practice <20 min physical activity on <3 days/week), not having participated in a weight-loss program (body weight changes <3 kg over the last three months), and not having any cardiovascular disease. This study was approved by the Ethics Committee on Human Research of the University of Granada (n°924) and by the Servicio Andaluz de Salud (Centro de Granada, CEI-Granada) [28]. The study protocol and the written informed consent were performed in accordance with the Declaration of Helsinki (revision of 2013).

Neck Circumference Assessment

Neck circumference (cm) was measured using an inextensible metallic tape over the thyroid cartilage and perpendicular to the longitudinal axis of the neck [29]. During the measurement, the participant was in an anatomical position, standing or sitting with the head in the Frankfort plane and shoulders relaxed.

Anthropometric and Body Composition Measurements

Body weight (kg) and height (m) were measured using a calibrated digital scale SECA (model 769, Hamburg, Germany) and a portable stadiometer brand SECA (model 213) respectively. The participants wore light clothing and no shoes during the measurements. BMI (kg/m^2) and Triponderal Mass Index (TMI, kg/m^3)

[30] were calculated. Waist circumference (WC) was measured in the minimum perimeter, at the end of a normal expiration, with the arms relaxed on both sides of the body. When the minimum perimeter could not be detected (such as in people who were overweight or had obesity), we took the measurements above the umbilicus, in a horizontal plane. Hip circumference was measured in the widest part of the gluteal region at the greater trochanter level [7]. We measured the perimeters of waist and hip (cm) twice with a plastic tape measure, and we used the average values for the analyses. We calculated the waist to hip ratio as well as the waist to height ratio.

In the same day in which the anthropometric measurements were performed, the participants underwent a Discovery Wi dual energy x-ray absorptiometry (Hologic, Bedford, Massachusetts, USA) scan in order to determine indicators of body composition, including fat mass, lean mass, and visceral adipose tissue (VAT). The participants underwent the scan with minimal clothing and not wearing any metal object. In addition, they were asked to stay as quiet and calm as possible during the scan time. Once the DXA scan was performed, we calculated the fat mass and lean mass indexes (FMI and LMI, respectively) [31] as fat or lean mass in kg divided by height in m^2.

Statistical Analysis

The distribution of the variables was verified using the Shapiro–Wilk test, skewness and kurtosis values, visual check of histograms, Q-Q, and box plots. The descriptive parameters of women and men were compared with an independent sample t-test (equal variances) or with the Welch's test (unequal variances).

Pearson correlations and multivariate stepwise forward linear regression analyses were used to examine (i) the association of neck circumference with anthropometric indicators (i.e., BMI, TMI, WC, W/hip, W/height) and body composition (i.e., FMI, LMI, VAT) and (ii) to examine the association of neck

circumference and other anthropometric indicators (i.e., BMI, TMI, WC, W/hip, W/height) with body composition (i.e., FMI, LMI, VAT) starting from the one with highest simple correlation in the univariable analyses. Semipartial correlation was used as a measure of the relationship between FMI, VAT and LMI with independent variables of multivariate model, after controlling for the effect that one additional variable had on one of those variables. The level of significance was set at $p < 0.05$. The statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS version 21.0, Chicago, IL, USA).

RESULTS

The main characteristics of the study participants are presented in Table 1.

Table 1. Characteristics of the participants.

	All (n = 119)	**Women (n = 82)**	**Men (n = 37)**	***p***
Age (years)	21.9 (2.3)	21.8 (2.2)	22.1 (2.4)	0.488
Weight (kg)	71.7 (16.4)	66.0 (11.6)	84.9 (18.0)	<0.001
Height (m)	1.69 (8.5)	1.65 (6.5)	1.77 (6.3)	<0.001
Neck circumference (cm)	34.3 (3.8)	32.3 (2.1)	38.8 (2.6)	<0.001
BMI (kg/m^2)	25.1 (4.6)	24.1 (4.0)	27.2 (5.3)	0.003
TMI (kg/m^3)	14.9 (2.7)	14.7 (2.5)	15.4 (3.0)	0.193
WC (cm)	81.6 (13.8)	77.4 (11)	90.1 (15.3)	<0.001
Waist/hip	0.85 (0.1)	0.80 (0.1)	0.85 (0.1)	<0.001
Waist/height	0.48 (0.08)	0.47 (0.07)	0.52 (0.09)	0.006
Fat mass (kg)	26.0 (8.8)	26.0 (7.5)	27.0 (11.3)	0.594
FMI (kg/m^2)	9.1 (3.0)	9.4 (2.7)	8.5 (3.5)	0.124
VAT (g)	348.5 (181.8)	307.4 (168.0)	439.4 (181.0)	<0.001
Lean mass (kg)	42.0 (9.9)	37.0 (5.0)	53.7 (7.6)	<0.001
LMI (kg/m^2)	14.6 (2.4)	13.5 (1.5)	17.2 (2.2)	<0.001

Values are means ± standard deviation. *p* for sex comparisons. BMI: Body mass index; FMI: Fat mass index; LMI: Lean mass index; TMI: Triponderal mass index; VAT: Visceral adipose tissue; WC: Waist circumference.

Figure 1 shows the correlations of neck circumference with anthropometric indicators and body composition by sex. NC was significantly and positively associated in both women and men (all $p \leq 0.002$)with BMI (rW = 0.70 and rM = 0.84, respectively), TMI (rW = 0.63 and rM = 0.80, respectively), WC (rW = 0.75 and rM = 0.86, respectively), VAT (rW = 0.74 and rM = 0.82, respectively), Waist/hip (rW = 0.51 and rM = 0.67, respectively), Waist/height

(rW = 0.68 and rM = 0.83, respectively), FMI (rW = 0.61 and rM = 0.81, respectively), and LMI (rW = 0.69 and rM = 0.68, respectively).

Figure 1. Association of neck circumference with indicators of anthropometry and body composition by sex (women: n = 82, men: n = 37). BMI: Body mass index; TMI: Triponderal mass index.

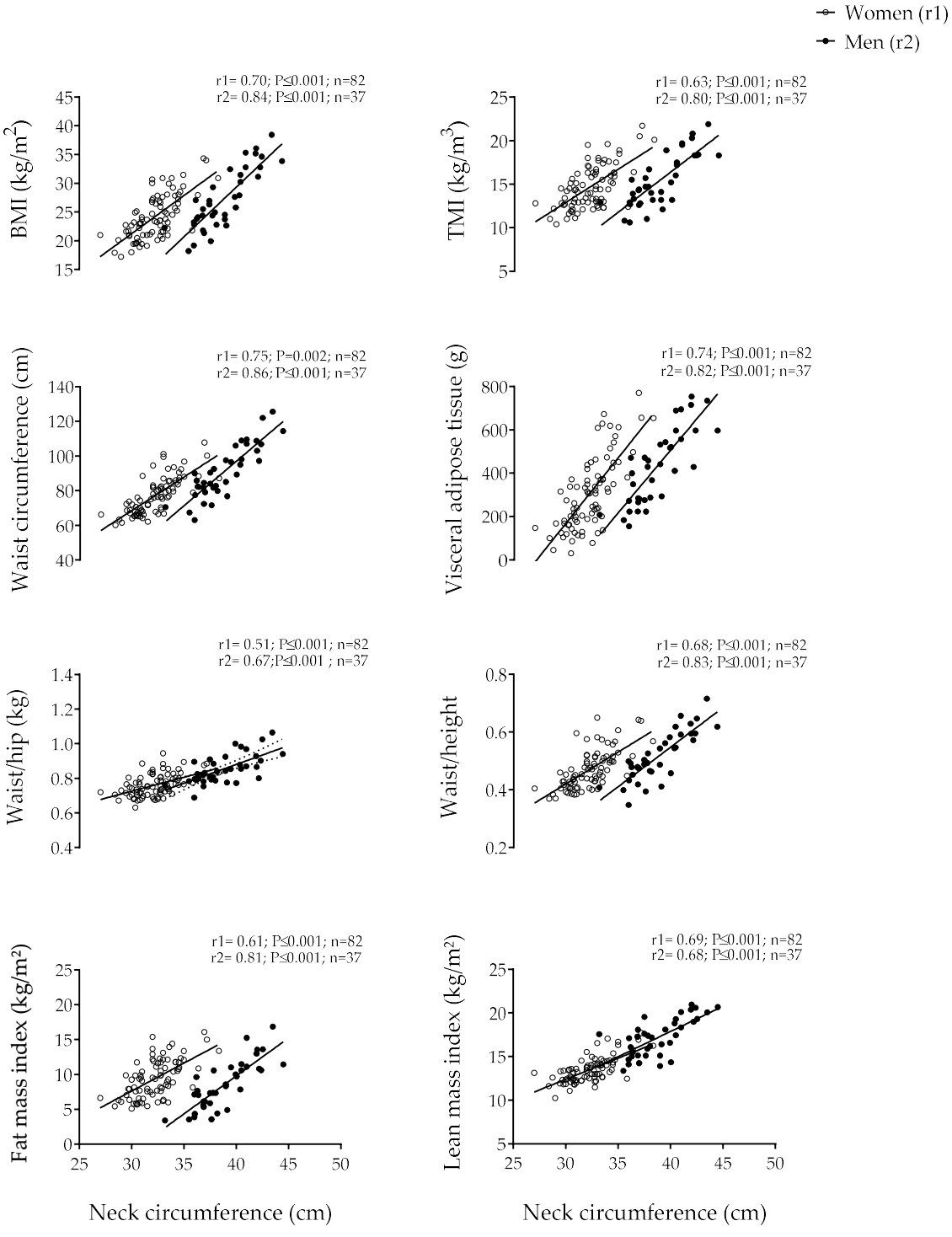
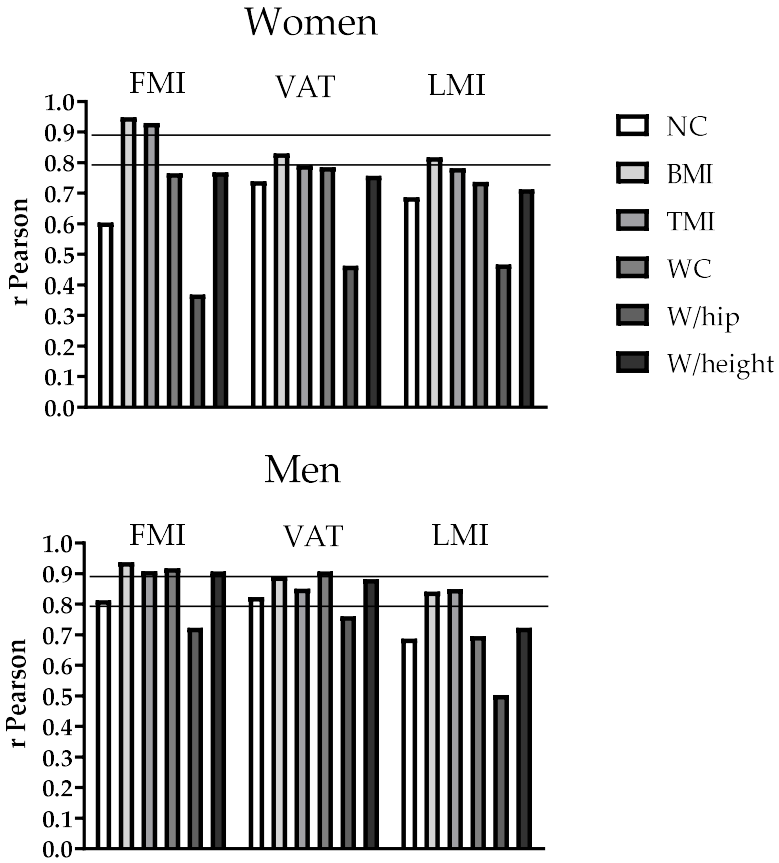


Figure 2 shows the correlations of NC and other anthropometric indicators with body composition measured by DXA in women and men. Pearson correlations of NC with indicators of body fat measured by DXA were consistently below 0.90. The association of neck circumference with indicators of body composition measured by DXA was weaker than that observed for BMI, TMI, WC and Waist/height, but not with Waist/hip.

Figure 2. Association of anthropometric indicators with body composition measured by Dual X-ray absorptiometry (DXA) in women (n = 82) and men (n = 37).



BMI: Body mass index; FMI: Fat mass index; LMI: Lean body mass index; NC: Neck circumference TMI: Triponderal mass index; VAT: Visceral adipose tissue; WC: Waist circumference; W/hip: Waist to hip ratio; W/height: Waist to height ratio.

In women, BMI (univariate model) was the strongest predictor of FMI (R^2 = 0.899, $p \leq 0.001$), of VAT (R^2 = 68.6, $p \leq 0.001$) and of LMI (R^2 = 66.7, $p \leq 0.001$). Neck circumference was associated with FMI, VAT and LMI independently of BMI (Table 2, multivariate model). However, in men, BMI (univariate model) was the strongest predictor of FMI (R^2 = 87.6, $p \leq 0.001$) followed by WC (multivariate model) with a variance explained of 5%. In addition, WC (univariate model) was the strongest predictor of VAT (R^2 = 82.0, $p \leq 0.001$) followed by BMI. Finally, TMI was the unique predictor of LMI (R^2 = 71.5, $p \leq 0.001$).

Table 2. Association of neck circumference , body mass index, triponderal mass index, waist circumference , waist to hip ratio and wa
composition measured by Dual X-ray absorptiometry (DXA) in women (n = 82) and men (n = 37).

						WOMEN				
		FMI				**VAT**				
		β	**95% CI**	**R^2**	**sr**	**β**	**95% CI**	**R^2**	**sr**	**β**
Univariable model										
-	NC	3.765 (0.554) ***	2.662 to 4.867	0.358		282.857 (28.706) ***	225.731 to 339.983	0.543		2.277 (0.270) *
-	BMI	3.025 (0.112) ***	2.801 to 3.248	0.899		162.546 (12.172) ***	138.323 to 186.769	0.686		1.391 (0.109) *
-	TMI	2.708 (0.120) ***	2.470 to 2.946	0.863		141.522 (12.208) ***	117.227 to 165.817	0.622		1.215 (0.108) *
-	WC	2.696 (0.253) ***	2.193 to 3.199	0.582		169.522 814.961) ***	139.749 to 199.295	0.611		1.384 (0.141) *
-	W/hip	1.281 (0.360) ***	0.565 to 1.997	0.126		98.527 (21.049) ***	56.638 to 140.416	0.205		0.864 (0.182) *
-	W/height	2.360 (0.220) ***	1.923 to 2.797	0.586		142.782 (13.746) ***	115.427 to 170.137	0.569		1.168 (0.128) *
Multivariable model										
-	BMI	3.269 (0.153) ***	2.965 to 3.573	0.904	0.735	119.758 (15.622) ***	88.664 to 150.853	0.734	0.439	1.125 (0.147) *
-	NC	−0.684 (0.298) *	−1.277 to −0.090		−0.079	119.875 (30.504) ***	59.159 to 180.592		0.225	0.746 (0.286)
						MEN				
Univariable model										
-	NC	5.115 (0.616) ***	3.864 to 6.367	0.653		270.139 (31.414) ***	206.366 to 333.912	0.670		2.717 (0.490) *
-	BMI	2.800 (0.175) ***	2.444 to 3.156	0.876		138.745 (12.002) ***	114.380 to 163.109	0.787		1.590 (0.172) *
-	TMI	2.775 (0.214) ***	2.340 to 3.211	0.822		135.489 (14.154) ***	106.754 to 164.224	0.716		1.640 (0.172) *
-	WC	2.839 (0.207) ***	2.420 to 3.259	0.839		146.521 (11.426) ***	123.325 to 169.717	0.820		1.360 (0.237) *
-	W/hip	2.475 (0.398) ***	1.667 to 3.284	0.511		135.740 (19.555) ***	96.040 to 175.439	0.567		1.086 (0.316)
-	W/height	2.777 (0.217) ***	2.337 to 3.217	0.819		140.969 (12.658) ***	115.273 to 166.666	0.774		1.399 (0.226) *
Multivariable model										
-	BMI	1.788 (0.400) ***	0.975 to 2.601	0.896	0.241	56.675 (26.110) *	3.612 to 109.738	0.837	0.146	1.640 (0.172) *
-	WC	1.144 (0.414) **	0.303 to 1.986		0.149	92.791 (27.033) **	37.855 to 147.728		0.231	
-	TMI									

Multivariate stepwise regression analysis to examine the association of anthropometric indicators with FMI, VAT and LMI. All the i
standardized (Z-score). β coefficient (standard deviation), 95% confidence interval (CI), adjusted coefficient of determination (R2), sr (
p-value are provided. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. BMI: Body mass index; FMI: Fat mass index; LMI: Lean body mass index; NC
Triponderal mass index; VAT: Visceral adipose tissue; WC: Waist circumference; W/hip: Waist to hip ratio; W/height: Waist to height r

DISCUSSION

In the present study we showed that neck circumference is associated with anthropometric indicators including BMI, TMI, or WC as well as with indicators of body fat measured by DXA such as FMI or VAT in a sample of young Spanish adults. In addition, we observed a positive association between neck circumference and LMI. It is of note that these associations appeared to be stronger in men than in women. The correlation of neck circumference with indicators of body composition measured by DXA was lower than that observed for other classic anthropometric indicators such as BMI and WC, which limits the value of neck circumference as a useful proxy of body composition parameters in young adults. However, in women, neck circumference was associated with all three measures of body composition independently of BMI and, therefore, it might be worth exploring in future studies its usefulness as a measure to use in combination with BMI.

Several studies examined the association between neck circumference and indicators of body composition in adults from different ethnic groups or races [11,12,23–27] of both sexes. Castro-Pinero et al. [32] showed weaker associations between neck circumference and FMI in girls 191 ($r = 0.494$, $p < 0.001$) and in boys ($r = 0.474$, $p < 0.001$) than in our study, most likely due to the fact that they estimated FMI from skin-fold thickness. Studies utilizing computer tomography as the reference method to asses body composition showed a positive and significant association of neck circumference with VAT and subcutaneous adipose tissue [11,23,26], whereas others only found a significant association with VAT [12,27]. On the other hand, studies assessing body composition by DXA [24,25] have shown that neck circumference is associated with the percentage of total body fat and abdominal fatness. Similarly, our findings showed that neck circumference was positively associated with FMI and VAT estimated by DXA. Thus, it

seems that neck circumference is a valid marker of total and central body fat in young adults, and that it could be implemented as an easy and practical measure. Interestingly, we observed that neck circumference was highly correlated to LMI, which was independent of BMI in women but not in men. To our knowledge, there are no studies investigating the association of neck circumference with LMI, which hamper between-studies comparisons.

Regarding anthropometric indicators, Ben-Noun et al. [22] showed, for the first time, that neck circumference was positively associated with BMI (women, r = 0.71; men, r = 0.83), WC (women, r = 0.85; men, r = 0.86), hip circumference (women, r = 0.56; men, r = 0.62), and waist/hip ratio (women, r = 0.87; men, r = 0.66) in adults. Later studies found similar results in Turkish [33], Pakistani [13], and Chinese [18,34] populations. In agreement with these studies, we observed that neck circumference was positively associated with BMI and TMI, and with anthropometric measures related to body fat distribution (i.e., WC, Waist/hip and Waist/height) in a sample of young Spanish adults.

Although neck circumference is an anthropometric indicator at least as simple as BMI and easier than WC in patients with weight excess, it might not add new information on body composition compared with other classic anthropometric indicators. We observed that the association of neck circumference with FMI, VAT, and LMI was weaker than that observed for BMI, TMI, WC and WC/height. In women, NC slightly improved the prediction of LMI, VAT and FMI beyond BMI. Future studies should explore its usefulness as a measure to use in combination with BMI. Assyov et al. [35] showed that WC was the best anthropometric measure to predict the distribution of adipose tissue measured by means of Body Impedance Analyse (BIA) in men and women with obesity (45–70 years old). Similar results were found by Joshipura et al. [36] in overweight or obese individuals

(40–65 years old), showing that BMI and WC were better correlated with body fat percentage (BIA) than neck circumference. It is however relevant that neck circumference seems to be more strongly associated with cardiovascular disease risk factors than other anthropometric indicators such as BMI or WC [35,36]. Consequently, although the available evidence points out that neck circumference might not be the best marker of body composition, its role as a predictive and easy tool to assess other cardiovascular disease risk factors should be further considered.

The cross-sectional nature of this study prevents us from determining any causality in the results. Our results are limited to young adults, and, therefore, whether neck circumference is a valid marker of body composition in older adults and people with cardiometabolic disease are not known. Furthermore, although DXA is a valid and extensively used method to assess body composition, further studies should consider the use of reference methods such as computed axial tomography or magnetic resonance imaging. In addition, our findings are limited by the sample size, and the differences of strength of the association between women and men could be driven for the differences of body composition and not for the sex. The present study is exploratory, without external validation.

CONCLUSIONS

In conclusion, neck circumference is associated with anthropometric indicators such as BMI and WC as well as with indicators of body composition measured by DXA (FMI, VAT, LMI), but the results indicate that it is not a better predictor of total and central body fat than other classic anthropometric markers as BMI or WC in young healthy adults. Taken together, the present study provides no indication for neck circumference as a useful proxy of body composition parameters in young adults, yet future studies should explore its usefulness as a measure to use in combination with BMI, especially in women.

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Study 2

Neck circumference is associated with adipose tissue content in thigh skeletal muscle in overweight and obese premenopausal women

ABSTRACT

Background: Neck circumference (NC) has been proposed as a simple and practical tool, independently associated with cardiometabolic risk factors. However, the association of NC with inter-muscular adipose tissue (IMAT) is still to be determined.

Aims: We aimed to examine the association of NC with thigh IMAT, and visceral adipose tissue (VAT) measured with computed tomography (CT) in overweight/obese women.

Material and Methods: 142 premenopausal overweight and obese Caucasian women participated in this cross-sectional study. NC was measured with an inextensible metallic tape above the thyroid cartilage according to International Society for Advancement of Kinanthropometry protocol. Thigh IMAT and VAT volumes were measured with a single cross-sectional CT. Regarding the covariates, fat mass (FM) was assessed with dual-energy x-ray absorptiometry and physical activity was objectively measured with accelerometry.

Results: NC was positively associated with thigh IMAT and VAT volumes (standardized β coefficient: β= 0.45, P-value=≤0.001, β= 0.60, P=≤0.001; respectively), which persisted after adjusting for age, height, overall FM or moderate-to-vigorous physical activity.

Conclusions: Our findings show that NC is associated with thigh IMAT volume in overweight and obese premenopausal Caucasian women, regardless of the amount of lower-body fatness. These results suggest underscoring the relevance of NC as a marker of adipose tissue content in thigh skeletal muscle.

Keywords: body composition, obesity, cardio-metabolic risk, intermuscular adipose tissue; intramyocellular lipid content; visceral adipose tissue.

INTRODUCTION

Obesity is associated with significantly higher all-cause mortality in almost two thirds of the adult population in developed countries (1). Although the deleterious effects of greater adiposity are well documented, obesity-related metabolic consequences are more associated with regional body fat distribution, ectopic fat deposition, and cellular infiltration, rather than absolute quantity (2; 3). An increased upper-body fat has been associated with adverse metabolic complications, in particular the visceral adipose tissue (VAT) located in the thoracic, abdominal and pelvic cavities (4; 5). In addition, a higher intermuscular adipose tissue (IMAT) located between muscle groups and beneath the fascia (6) is related to greater risk of all-cause mortality. Each one-standard deviation increase in IMAT (~6.8% greater IMAT) is associated with a 40% greater mortality risk over a 10-year period (7). IMAT volume is higher in individuals with obesity and type 2 diabetes (8; 9) and is associated with high levels of inflammation, insulin resistance, obesity, sarcopenia, and lower physical performance in adults and the elderly population (10; 11). Recently, Bergia et a. showed that greater IMAT in the thigh is a better predictor of cardiometabolic risk than greater IMAT in the calf in adults who are overweight and obese. (11). Interestingly, IMAT is a unique ectopic adipose depot involved in a range of adverse health outcomes similar to VAT (12).

In the body, adipose tissue two compartments are clearly distinguished: Subcutaneous adipose tissue (SAT) (80% of all body), and VAT (intra-abdominal fat) (13). IMAT have been definite as part of SAT depot located between muscle groups and beneath the muscle fascia (14). Advances in imaging technology have enabled the identification of IMAT and VAT thought the use of highly sensitive imaging techniques as computed axial tomography (CT) scan and magnetic resonance imaging (MRI) (15).

Recently, emerged evidence reveals that volume of neck adipose tissue is independently associated with cardiovascular risk factors, metabolic syndrome prevalence and long-term mortality (16-20). Consequently, neck circumference (NC) has been proposed as a novel upper-body fatness anthropometric measurement for weight management in children and adults (21; 22), due to the associated low cost and feasibility (23; 24). Previous studies observed a strong association between NC, total abdominal fat, SAT and VAT using CT or MRI (17; 25; 26). However, the association of a larger NC with a higher IMAT is still to be determined. Still, IMAT determination is limited by the radiation exposure associated with CT and by the relatively high cost of magnetic resonance imaging (MRI) analysis precluding its use at clinical settings. Therefore, simple anthropometric indicators, such as NC, waist circumference (WC), body mass index (BMI) but also fat mass (FM) or fat mas index (FMI) will be more practical to predict cardiovascular risk factors at clinical settings as alternatives to estimate IMAT. Nevertheless, the usefulness of these practical anthropometric measures and indexes of adiposity as surrogates of IMAT at the tissue level is still unclear.

Simple and feasible anthropometric measures such as BMI and WC have been explored as markers of IMAT (27-30). The usefulness of simple, low cost and feasible anthropometric indicators, such as NC, over other commonly used overall or central fat mass (FM) indexes has not been explored as potential markers of IMAT. Therefore, in the present investigation we aimed to examine the association of NC and other simple measures such as WC, BMI, FM and FMI with thigh IMAT, and VAT measured with computed tomography (CT) in overweight/obese women.

MATHERIALS AND METHODS

Research design and participants

The baseline data of 142 premenopausal overweight and obese Caucasian women participating in a randomized controlled trial (Clinical Trials, ID: NCT00513084) were used. The inclusion criteria were being >24 years, >24.9 kg/m2 BMI, not being pregnant, without any cardio-metabolic disease, not being on any medications or not having had an intervention that affects the weight or body composition. All the assessment was performed in the Faculty of Human Movement, Technical University of Lisbon, from 2002 till 2003. The informed consent was signed by the participants. The study protocol was performed according to the principles of the Helsinki Declaration and approved by the Human Research Ethics Committee of the University of Lisbon.

Procedures

Anthropometry

With the participants in anatomical position, standing or sitting with the head in the Frankfort plane and shoulders relaxed, NC was measured with a plastic tape calibrated weekly over the thyroid cartilage, and perpendicular to the longitudinal axis of the neck, according to International Society for Advancement of Kinanthropometry protocol(31). Based on test-retest in 10 participants, the coefficient of variation (CV) for the NC was 0.45%. Weight and height were measured using a scale (SECA, Hamburg, Germany) and a stadiometer (Seca, Hamburg, Germany) previously calibrated and WC was measured according to Lohman et al.'s (32) procedures. All measurements were made in duplicate, using the mean for the analysis. BMI was calculated by dividing body weight by the squared height in meters (kg/m2).

Body composition

Single cross-sectional CT. Using a CT scan (Somaton Plus; Siemens, Sorheim, Germany), participants were assessed in supine position with arms extended above their head. A single cross-sectional CT at the L4-L5 intervertebral space image was acquired to measure the VAT area. The boundary between VAT and abdominal subcutaneous adipose tissue was defined using the abdominal and oblique muscles in continuity with the deep fascia of the paraspinal muscles and the anterior aspect of the vertebral body (33). Cross-sectional CT thigh images were also obtained using contiguous 7-mm–thick cross-sectional images of both legs obtained between the inferior ischial tuberosity and the superior border of the patella. The IMAT region was measured between muscle groups and underneath the fascia. The IMAT volume (cubic centimeters) identified in each image was calculated by multiplying the image thickness (7mm) by the tissue area (square centimeters), and IMAT volume (litters) was then converted to mass units (kilograms) multiplying the volume by the assumed constant of fat density (0.92 kg/L) (34).

All images were obtained using 120 kVp, 480 mA, and 512 × 512 matrix with a 48-cm field of view. CT data were analysed by specific software (Slice-O-matic, Version 4.2, Tomovision, Montreal, Canada) based on image morphology. A combination of watershed techniques and edge detection filters was employed. Different tissues were identified using boundaries in Hounsfield Units (HU) set to –29 to +150 for muscle, –190 to –30 for IMAT and subcutaneous adipose tissue, and –150 to –50 for VAT (35).

Dual Energy X-ray Absorptiometry (DXA). Total fat mass, fat-free mass, lean soft tissue, appendicular lean soft tissue mass, leg fat and trunk fat were measured with a pencil beam mode DXA (QDR-1500 Hologic, Waltham, Mass, USA). The equipment measures the attenuation of X-rays pulsed between 70 and 140 kV synchronously with the line frequency for each pixel of the scanned image.

Following the protocol of DXA described by the manufacturer, a step phantom with six fields of acrylic and aluminium of varying thickness and known absorptive properties was scanned to serve as an external standard for the analysis of different tissue components. The same technician positioning the participants performed the scans and executed the analysis according to the operator's manual using the standard analysis protocol. Based on test–retest using 10 participants, the coefficients of variation (CV) in our laboratory of FM is 2.9%. FMI was calculated as FM in kg divided by squared height in m.

Physical Activity Assessment

All participants were asked to use an accelerometer (ActiGraph, GT1M model, Fort Walton Beach, Florida, USA), worn on the right hip near the iliac crest during 7 consecutive days including weekend days (36). The delivery and reception of the accelerometers, as well the explanation of its use, were personally carried out with the participants (Ward et al. 2005). The devices were activated on the first day at 07:00, and data were recorded in 10-s epochs. The device activation and data download were performed using the software Actilife Lifestyle (ver. 3.2). Processing was performed using the software MAHUffe (ver. 1.9.0.3; available at www.mrc-epid.cam.ac.uk) from the original downloaded files (*.dat). For the analyses, a valid day was defined as having 600 or more minutes (10 h) of monitor wear, corresponding to the minimum daily use of the accelerometer (37). Apart from accelerometer nonwear time (i.e., when it was removed for sleeping or water activities), periods of at least 60 consecutive min of zero activity intensity counts were also considered as nonwear time. The amount of activity assessed by accelerometry was expressed as the number of minutes per week spent in moderate-to-vigorous physical activity (MVPA) using the cutoff values proposed by Troiano (38) for moderate intensity (2020–5998 counts/min, corresponding to 3–5.9 METs) and vigorous intensity (≥5999 counts/min, corresponding to ≥6 METs).

Statistical analyses

Descriptive statistics were performed to describe the characteristics of the study participants. Pearson correlation analyses were used to examine the relationship of NC and other anthropometric indicators with thigh IMAT and VAT.

Multivariate lineal regression was performed to examine the unadjusted associations of NC, BMI, WC, FM and FMI with thigh IMAT and VAT indicators (model 1) or to adjust for age (model 2). Statistical analyses were performed using the SPSS software version 21.0 (SPSS, Chicago, IL, USA) and statistical significance was set at P-value <0.05.

RESULTS

Table 1 displays the descriptive characteristics of the participants of the study. The accelerometer wear mean time was 13.8± 1.3 h/day and participants spend the 71% of their waking time (16.91±1.5 h/day) in sedentary behavior.

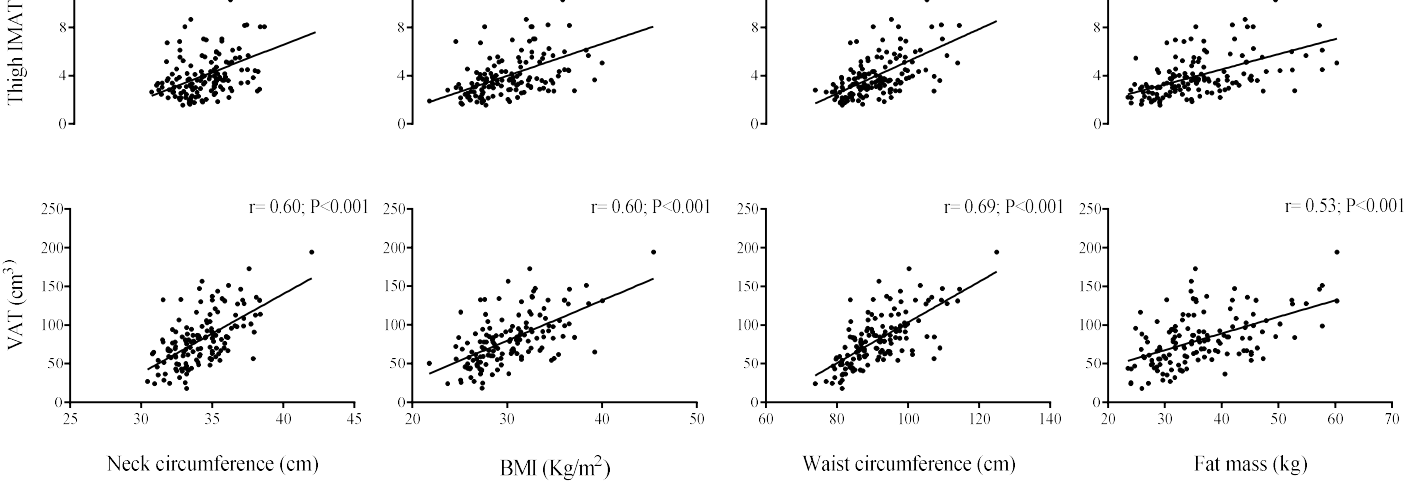
Table 1. Descriptive characteristics of the study participants.

Variables	N	Mean ± DS
Age (years)	142	38.1 ± 5.8
Weight (kg)	142	78.0 ± 10.8
Height (m)	142	1.6 ± 0.1
NC (cm)	142	34.2 ± 1.9
BMI (kg/m^2)	142	30.2 ± 3.8
WC (cm)	142	91.4 ± 8.7
Thigh IMAT volume (cm^3)	142	4.0 ± 2.0
Thigh IMAT area (cm^2)	142	5.8 ± 2.8
VAT (cm^3)	142	80.8 ± 32.9
VAT (cm^2)	142	115.4 ± 47.0
Legs fat mass (kg)	142	13.2 ± 3.1
Trunk fat mass (kg)	142	18.0 ± 4.8
Total fat mass (kg)	142	36.2 ± 8.1
Appendicular lean soft tissue mass (kg)	142	16.0 ± 2.3
Total fat free mass (kg)	142	41.2 ± 4.7
Lean soft tissue (kg)	142	38.7 ± 4.4
Waking time (min/day)	109	827.4± 75.8
Sedentary time (min/day)	109	1015.2 ± 89.0
Light physical activity (min/day)	109	381.3 ± 84.6
Moderate physical activity (min/day)	109	34.3 ± 19.0
Vigorous physical activity (min/day)	109	1.46 ± 4.1
Moderate-vigorous physical activity (min/day)	109	35.8 ± 20.4

Values of sample size together with mean ± standard deviation, are provided. BMI: Body mass index; IMAT: Intermuscular adipose tissue; NC: Neck circumference; VAT: Visceral adipose tissue; WC: Waist circumference.

Figure 1 illustrates the significant correlations between NC, BMI, waist circumference, FM and FMI with t

Figure 1. Association of neck circumference (NC), Body mass index (BMI), Waist circumference (WC) and Fat mass index (F
adipose tissue (IMAT)and Visceral adipose tissue (AT) in premenopausal Women (n=142). Pearson correlation coefficients and



In table 2, the unadjusted (Model 1) or adjusting for age (Model 2) show that NC, BMI, waist circumferenc
associated with thigh inter-muscular adipose tissue and visceral adipose tissue in premenopausal women
in the unadjusted analyses (Model 1) were positively associated with thigh IMAT, and VAT (Standardized

Pvalue ≤0.001, β= 0.60, P=≤0.001; respectively). When linear regression analyses were additionally adjusted
significantly associated with thigh IMAT (β= 0.41, P=≤0.001). Similarly, NC was a significant predictor of V
(β= 0. 54, P=≤0.001). We repeated the analyses adjusting for height, FM (kg) and MVPA and the results did
shown).

Table 2. Association of neck circumference (NC), Body mass index (BMI), Waist circumference (WC) Fat mass (FM) and Fat ma
inter-muscular adipose tissue (IMAT)and Visceral adipose tissue (VAT) in premenopausal Women.

	Thigh IMAT (cm3)		**VAT (cm^3)**	
N=142	Model 1	Model 2	Model 1	Model 2
NC (cm)	0.45 **	0.41 **	0.60 **	0.54 **
BMI (kg/m^2)	0.51 **	0.47 **	0.60 **	0.54 **
WC (cm)	0.59 **	0.56 **	0.69 **	0.64 **
FM (kg)	0.52 **	0.49 **	0.53**	0.49**
FMI	0.51 **	0.47 **	0.58 **	0.52 **

Multivariate lineal regression was performed to examine the association of NC, BMI, WC, FM and FMI with Thigh IMAT and VA
for any confounder (model 1) or adjusting for age (model 2). Standardized β coefficient, and P-value are provided. Statistically
P≤0.01, **P≤0.001. BMI: Body mass index, FM: Fat mass, FMI: Fat mass index, IMAT: Intermuscular adipose tissue, NC: N
circumference, VAT: visceral adipose tissue.

DISCUSSION

The current investigation shows, for the first time, a positive association of NC with thigh IMAT volume in overweight and obese premenopausal Caucasian women. These findings suggest that NC could be a valid measure of ectopic fat deposition as well as abdominal body fat in overweight and obese premenopausal women.

Our results extend previous findings that underlined (the fact) that NC has as a simple and practical indicator of adiposity (21; 39) and is positively associated with VAT measured with MRI and CT (16; 17; 25; 40). In addition, we observed that, after adjusting for age, NC, BMI, WC, FM, and FMI remained significant predictors of VAT. Interestingly, NC was similar to other indicators as VAT predictor. As no previous investigations explored the association between NC with thigh IMAT, no comparisons can be provided. However it should be underscored the role of intermuscular depots of fat located in the thigh as a relevant predictor of cardiometabolic risk factors in adults who are normal, overweight or obese (11; 41). Taken together, these findings uncover the potential role of NC as a subrrogate of a patogenic neck adipose tissue due to a greater flow of systemic free fatty acids (FFA), that could partly explain the cardiometabolic risk missed by VAT (17; 40). In fact, previous evidence has showed that elevated FFA concentrations reproduce the metabolic abnormalities of obesity and that the upper body fat increased in women with obesity would be the most important contributor in the systemic FFA release (42).

In this investigation, we observed that anthropometric indicators and FM markers were correlated with ectopic fat deposition, being this association particularly higher for WC and FM. In additon, we showed that an increase in adjusted, BMI, WC, FM and FMI markers explained an increase of 0.5-0.6 units in IMAT, while for NC this assocation had a lower variation (0.4 units of IMAT). These results extend previous observations between anthropometric markers

with body fat as the outcome of interest (23; 43). In a sample of overweight and obese adults where body-fat percentage was measured by a bioelectrical impedance analysis (BIA), Joshipura et al. (43) showed a lower coefficient of correlation of NC (r=0.45, P= <0.001) compared to WC (r= 0.62, P= <0.001) and BMI (r=0.65, P= <0.001), even after adjusting for age, gender, smoking status, and physical activity. Similarly, Assyov et al. (23) in a sample of adults that included pre and postmenopausal women with obesity found that WC (r=0.60, P=< 0.001) showed a higher association with adiposity compared to NC (r=0.43, P< 0.001). Nevertheless, NC presents a better association in the assessment of metabolic health compared to WC, specifically with fasting plasma glucose, fasting insulin, uric acid, HDL cholesterol and serum triglycerides (26; 43). Recently, similar results have been shown by Borel et al. in 305 women with severe obesity where NC was a better anthropometric marker to identify a high cardiometabolic risk compared to WC and BMI. (18). The interesting observation is that FMI presented a slighly lower ability to predict VAT and IMAT in the adjusted model compared to BMI. This is in line with Ortega et al.'s findings, (44) who showed that BMI was a stronger predictor of CVD mortality than total adiposity markers, particularly, fat-mass percentage and FMI assessed using accurate methods.

It is important to underscore that the reference techniques used in this study to assess IMAT and VAT along with DXA measures for total and regional body composition determination strengthen the findings of this study. Also, the inclusion of accelerometry data to assess the role of habitual physical activity as a potential confounder in these findings was explored. Nevertheless, some limitations should also be addressed. The cross-sectional nature of this study does not establish causality, and a possible reverse causality between anthropometric measures and adipose tissues compartments should not be disregarded. Our findings can only be generalized to Caucasian overweight/obese premenopausal women.

In conclusion, we suggest that a larger NC is associated with a higher volume of VAT but also with a higher amount of ectopic fat deposition in the thigh skeletal muscle, underscoring the relevance of NC as an indicator of adipose tissue content in thigh skeletal muscle. Additionally, when WC, BMI or adiposity are not available or are invalidated (edema, abdominal distension or increase in lean mass) in the clinical practice, NC should be used as an alternative anthropometric measure to predict thigh IMATand VAT in overweight and obese premenopausal women due to its simplicity, feasibility and low cost.

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Study 3

Neck adipose tissue accumulation is associated with higher overall and central adiposity, cardiometabolic risk, and pro-inflammatory profile in young adults

ABSTRACT

Background: Neck adipose tissue (NAT) volume increases with general adiposity, with fat accumulating in different neck tissue compartments. In patients with certain malignant/benign tumours, the accumulation of NAT, and certain NAT distributions, have been associated with cardiometabolic risk (CMR). However, it is unknown whether the same relationships exist in healthy people, and whether NAT accumulation and distribution are related to the inflammatory status.

Material and Methods: In this cross-sectional study, 139 young healthy adults (68% women) underwent a computed tomography scan to quantify the volume of compartmental (i.e., subcutaneous, intermuscular and perivertebral) and total NAT at the height of vertebra C5. Anthropometric indicators were measured, and body composition determined using dual energy X-ray absorptiometry. Information on CMR factors (i.e., blood glycaemic and lipid markers, blood pressure and physical fitness) was also gathered, and a CMR score calculated. Several plasma cytokines and serum components of the innate immune system were measured to determine the inflammatory status.

Results: Compartmental and total NAT volumes were directly related to BMI, and lean, fat, and visceral adipose tissue (VAT) masses (all, $P \leq 0.05$). Larger compartmental (especially intermuscular) and total NAT volumes were directly associated with the CMR score, several CMR factors (i.e., glycaemic and lipid markers and blood pressure), and the C3, C4 and leptin concentrations. They were, however, inversely correlated with the CMR factors HDL-C and physical fitness, and with the adiponectin concentration (all $P \leq 0.05$). Several of these associations remained statistically significant ($P \leq 0.05$) after adjustment for BMI, body fat percentage or VAT mass. Overall, results did not change after applying False Discovery Rate correction.

Conclusions: NAT volume and its distribution among different tissue compartments, appears to be associated with the CMR and inflammatory profile of young healthy adults. Total NAT volume might be as valuable as VAT mass in terms of predicting CMR and inflammatory status.

Keywords: adipose tissue expandability, cardiometabolic diseases, inmunometabolic profile, neck fat accumulation, obesity, upper subcutaneous fat mass.

INTRODUCTION

Obesity is a disorder of the energy homeostasis system; in simple terms it is the result of energy intake being sustained above energy expenditure. Obesity is partly characterized by the limited expandability and dysfunction of adipocytes, an increased cardiometabolic risk (CMR), and a low-grade chronic pro-inflammatory status (1-3).

Traditional measures of obesity include the body mass index (BMI) and waist circumference (4) - factors with a bearing on cardiometabolic disease (5). Several studies have proposed that fat accumulation in the neck (measured as neck circumference [NC]) provides an indicator of upper body fatness - which is also associated with obesity and cardiovascular risk independent of BMI and visceral adipose tissue (VAT) mass (6; 7). Accordingly, experimental evidence has shown that the splanchnic adipose tissue accounts for only a small percentage of systemic free fatty acid (FFA) release, while the upper body fat contributes >50%, rendering it the largest contributor of all (8). All the evidence gathered to date supports the idea that the upper body fat, and particularly the NAT, might, at least partially, explain the obesity-related CMR not covered by the VAT (9-11).

Torriani et al. (9) showed that NAT increases with increasing adiposity, but that it follows a different pattern of accumulation across the neck subcutaneous, intermuscular and perivertebral compartments - a pattern that also differs between the sexes. Further, they showed that the accumulation of NAT among these compartments correlated differently with CMR factors and the prevalence of metabolic syndrome. Similarly, Rosenquist et al. (10) observed that upper body subcutaneous fat, estimated by multidetector computed tomography imaging, was positively related to CMR factors and other measures of adiposity. Recently, Tal et al. (12) observed that a larger NAT volume relative to height, was directly associated with long-term mortality, independent of age and sex, as well as with the presence of type 2 diabetes mellitus. Current evidence would therefore seem

to indicate that fat accumulation in the neck might play a role in the development of cardiometabolic abnormalities (9). It should be noted, however, that all the above-mentioned studies involved cohorts of middle-aged (55-62 years old) patients who were retrospectively assessed for clinical purposes (i.e., malignant/benign tumours, suspected cardiovascular accidents, or vascular calcification) and included no estimate of physical fitness (a major CMR factor) (13). It therefore remains to be seen whether these findings apply to young, healthy, sedentary adults, or whether a larger compartmental or total NAT accumulation are related to other CMR factors.

It is well known that inflammatory activity appears early in adipose tissue expansion, and exists during chronic obesity (14-16). This can lead to maladaptive responses such as fibrosis, hypoxia and necrosis (among others) in adipose tissue, and negatively influence metabolic homeostasis (17). For instance, local changes in VAT deposits induced by adipocyte expansion are related to a low-grade systemic inflammation status characterized by elevated concentrations of circulating pro-inflammatory markers (16; 18-20). Whether specific NAT compartments are associated with a more inflammatory profile is unknown. The aim of the present work was, therefore, to examine the relationship between compartmental/total NAT and overall/central adiposity, CMR, and inflammatory markers in young, healthy, sedentary adults.

MATERIALS AND METHODS

Study subjects and ethics statement

A total of 139 young, healthy adults (95 women), all belonging to the ACTIBATE study population (ClinicalTrials.gov, ID: NCT02365129) (21), took part in this cross-sectional study. All subjects had to be age 18-25 years old, have a sedentary lifestyle (i.e., undertaking <20 min moderate-vigorous physical activity <3 days/week at baseline), to be a non-smoker, take no medication, have had a stable body weight over the last 3 months (changes <3 kg), to have no cardiometabolic disease (e.g., hypertension or diabetes), and to have no first-degree relative history of cancer. 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. All work was performed in accordance with the Declaration of Helsinki (2013 revision); all subjects gave their written informed consent to be included. All assessments were made in Granada (Spain).

Procedures

18F-FDG-PET/CT assays

18F-fluordeoxyglucose positron emission tomography combined with computed tomography (18F-FDG-PET/CT) was used to quantify NAT volume and distribution (these analyses were completed over eight dates distributed between October and December of 2015 and 2016, i.e., four per year, with one test per subject). The subjects all confirmed they had met the requirements 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) having not consumed any alcoholic or stimulant beverages in the previous 6 h or taken any drugs that might affect the peripheral circulation in the last 24 h. The subjects were then invited to dress in standardized clothing and to void their bladders.

Since the original aim of the ACTIBATE study (21) was to detect the volume and activity of brown adipose tissue (BAT), participants were submitted to a personalized cooling protocol prior to the 18F-FDG-PET/CT scan in order to stimulate BAT metabolic activity, as previously explained (22). After 60 min of following this personalized cooling protocol, a bolus of 18F-FDG (180.6 ± 5.8 MBq ≈2.9 MBq/kg). was injected. One hour later, the subjects underwent PET/CT using a Siemens Biograph 16 PET/CT scanner (Siemens, Erlangen, Germany). After lying down on a flat table (supine position), with a thin pillow below their heads to make them feel more comfortable, a low dose CT scan (120 kV) was performed for attenuation correction and anatomic localization. Immediately thereafter, one static acquisition of 2 PET bed positions (6 min each) was performed from the atlas vertebra to the mid chest region.

Neck measurements

Quantification of neck adipose tissue

For the main study purpose, only the CT component of the PET/CT was used. The CT scans were analysed using the Beth Israel plugin for FIJI software http://sourceforge.net/projects/bifijiplugins/ by the same researcher (JMPG). To determine the NAT volume and the distribution of fat across the different NAT compartments, several regions of interest (ROIs) were outlined at the level of C5 – the height at which NC measurement is usually performed (i.e., the level of laryngeal prominence (9))- , using a 3D-axial technique. NAT volumes were calculated for the chosen ROIs by determining the number of pixels within the radiodensity range of -300 to -10 Hounsfield Units (HU). An extended description of how the ROIs were drawn and analysed for each NAT specific compartment can be found in the supporting Information. Briefly, the three main NAT compartments were defined as:

i) subcutaneous NAT: adipose tissue in the posterior neck, between the skin and deep cervical fascia (see Figure 1F).

ii) 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 (see Figure 1F).

iii) perivertebral NAT: adipose tissue interspersed between the muscles surrounding vertebrae C5 (see Figure 1F).

In addition, using a 3-D sagittal view, another ROI was outlined at the height of C5 to determine the total NAT (i.e., with no compartmental differentiation) and lean tissue volume (23). This ROI was drawn parallel to the body's sagittal axis, and included the entire neck length and width from the upper to the lower part of vertebra C5. Pixels were deemed to represent NAT when they fell within the same radiodensity range, while those within the range of -9 to 150 HU range were deemed to represent lean tissue (including skeletal muscle tissue, blood vessels and certain internal organs) (23).

Neck circumference

NC was measured using an inextensible metallic tape over the thyroid cartilage, perpendicular to the longitudinal axis of the neck (24). During this measurement subjects were in an anatomical position, standing or sitting with the head in the Frankfort plane and the shoulders relaxed.

Anthropometry and body composition

Subject weight and height were respectively measured using a model 769 calibrated digital scale and a portable model 213 stadiometer, both from SECA (Hamburg, Germany). BMI was determined as body weight (kg)/height squared (m2). On the same day, subjects underwent dual energy x-ray absorptiometry

using a Discovery Wi device (Hologic, Bedford, Massachusetts, USA) to determine fat mass, lean mass, and VAT mass. We additionally determined the lean and fat mass and percentage of the trunk and appendicular regions (i.e., arms and legs) for secondary analyses, given the fact that appendicular body composition represents a stronger predictor for physical fitness than whole body lean mass, in diseases such as heart failure (25).

Cardiometabolic and inflammatory profile

CMR and inflammation markers were normally assessed within 3 weeks of the 18F-FDG-PET/CT assessment. Subjects came to our centre for the extraction of blood samples after an overnight fast (10–14 h).

Glycaemic, lipids markers and HOMA index

Serum glucose, total cholesterol, high density lipoprotein-cholesterol (HDL-C) and triglycerides were assessed following standard methods using an AU5832 automated analyzer (Beckman Coulter Inc., Brea CA, USA). Low density lipoprotein-cholesterol (LDL-C) was estimated as: [total cholesterol – HDL-C – (triglycerides/5)] (all in mg/dL) (26). Serum insulin was measured using the Access Ultrasensitive Insulin Chemiluminescent Immunoassay Kit (Beckman Coulter Inc., Brea CA, USA). The homeostasis model assessment of insulin resistance (HOMA) index was calculated as (insulin [μU/mL] x glucose [mmol/L]/22.5 (27).

Systolic and diastolic blood pressure

An Omron M6 upper arm blood pressure monitor (Omron Healthcare Europe B.V. Hoofddorp, The Netherlands) was used to determine the systolic and diastolic blood pressure, with subjects seated and relaxed. Measurements were taken at three time points, and the mean determined for use in later analyses.

Physical fitness: muscular strength

Handgrip strength was determined using an adjustable grip TKK 5101 Grip - D hand dynamometer (Takei, Tokyo Japan). Subjects were asked to squeeze gradually and continuously for a few seconds, and were encouraged to do their best when performing the tests. All tests were performed using the optimal grip-span (28). Each subject performed two attempts with each hand, with the arm fully extended and maintaining the trunk erect. The maximum score for each hand was recorded in kilograms and the mean score of the left and right hand used in analyses. Muscular strength relative to body weight and lean body mass was also calculated.

Physical fitness: cardiorespiratory fitness

Subjects' maximum oxygen consumption (VO2max) was determined via a maximum exercise test using a Pulsar treadmill (H/P/Cosmos Sport & Medical GMBH, Nußdorf, Germany), based on the modified Balke protocol (29). O2 consumption and CO2 production were measured by indirect calorimetry using a CPX Ultima CardiO2 cart (Medical Graphics Corp, St Paul, USA) and a Model 7400 oronasal mask (Hans Rudolph Inc., Kansas City, MO, USA) equipped with a Prevent™ metabolic flow sensor (Medgraphics Corp., St. Paul, MN, USA). The criteria for achieving VO2 max were: a respiratory exchange ratio of ≥1.1, a plateau in VO2 (change of <100 mL/min in the last three consecutive 60 s stages), and a heart rate within 10 beats/min of the age-predicted maximum (208–0.7 x age) (Pallarés & Morán-Navarro, 2012). When no plateau in VO2 was reached, VO2 peak was obtained, and taken to represent cardiorespiratory fitness. The latter variable was also recorded relative to body weight and lean body mass.

Cardiometabolic risk score

A CMR score based on variables included in the diagnostic of Metabolic Syndrome (30) was computed, including the subject's waist circumference, blood

pressure, plasma glucose, and HDL-C and triglyceride concentrations. Each variable was standardized as follows: standardized value = (value - mean)/standard deviation. The HDL-C standardized values were multiplied by -1 to represent increasing values as directly proportional to the risk score. The final score was determined as the sum of the five standardized scores divided by five.

Pro and anti-inflammatory markers

C-reactive protein, C3, C4, and β-microglobulin 2 concentrations were measured by immunoturbidimetric assay, employing the same AU5832 automated analyser as above. Interleukin (IL)-2, IL-4, IL-6, IL-7, IL-8, IL-10, IL-17a, interferon gamma (IFNɣ) and tumor necrosis factor alpha (TNF-α) were determined using a MILLIPLEX MAP Human High Sensitivity Cytokine Panel from the Luminex Corporation (Missouri, USA; Catalogue # HSCYTMAG-28SK). Leptin and adiponectin concentrations were measured using the MILLIPLEX MAG Human Adipokine Magnetic Bead Panel 2 (Catalogue # HADK2MAG-61K) and the MILLIPLEX MAP Human Adipokine Magnetic Bead Panel 1 (Catalogue # HADK1MAG-61K) respectively, both from the Luminex Corporation. Intra-assay CVs can be found in the Supporting Information (31).

Statistical analyses

Descriptive statistics for continuous and categorical variables were recorded for all subjects. All variables related to NAT, and the cardiometabolic (except muscular and cardiorespiratory fitness) and inflammatory profiles, were square root-transformed to render their distributions closer to normal. All the analyses were performed separately for women and men given their important metabolic, phenotypic and NAT distribution differences, and given the influence of the interactions sex x body composition/ CMR/ inflammatory profile on NAT variables. The Kruskal-Wallis test was used to compare neck measurements

across BMI categories. Pearson correlations were calculated to examine the relationship of compartmental and total NAT volume with NC, and to examine the relationship between neck measurements and body composition variables. Pearson correlation analysis was also conducted to examine the associations between neck measurements and VAT mass with CMR and the inflammatory profile. Adjustments for multiple comparisons were performed with the Benjamini-Hochberg procedure (False Discovery Rate-FDR- correction), to control the overall type I error rate (32). This procedure was applied for the main analyses. All-statistical analyses were performed using SPSS software version 21.0 (SPSS, Chicago, IL, USA). Significance was set at $P<0.05$.

RESULTS

In the present study we initially included a total of 139 participants (see Flow Chart, Supporting Information) (31), all of whom underwent a 18F-FDG-PET/CT scan in order to measure and quantify adipose tissue in the upper body region. Nevertheless, after checking and analysing all CT scans to measure and quantify NAT, several participants were excluded for each specific analysis (see Supporting Information for detailed information) (31). The sample size varies for the different variables (e.g., NAT measures, body composition, cardiometabolic or inflammatory parameters). Therefore, in order to make the maximum use of the data, we performed the analyses with all valid data on the specified measures. The main characteristics of the whole study cohort are presented in Table 1.

Table 1. Descriptive characteristics of the study subjects.

	ALL		WOMEN		MEN	
Age (years)	22.1 (2.2)	139	22.0 (2.1)	95	22.3 (2.2)	44
Anthropometry and body composition						
Weight (kg)	70.4 (16.1)	139	64.5 (11.9)	95	83.2 (16.7)	44
Height (m)	1.68 (8.53)	139	1.64 (6.6)	95	1.76 (6.6)	44
BMI (kg/m²)	24.8 (4.5)	139	23.8 (3.9)	95	26.9 (5.1)	44
Normal-weight percentage (%)	60.4	84	67.4	64	45.5	20
Overweight percentage (%)	25.2	35	23.2	22	29.5	13
Obese percentage (%)	14.4	20	9.5	9	25.0	11
Waist circumference (cm)	80.7 (13.9)	136	76.3 (10.8)	92	90.0 (15.0)	44
Waist-hip ratio	0.8 (0.1)	136	0.8 (0.1)	92	0.9 (0.1)	44
Lean mass (kg)	41.6 (9.5)	139	36.4 (5.0)	95	53.0 (6.4)	44
Fat mass (kg)	24.9 (8.9)	139	24.5 (7.7)	95	25.5 (11.2)	44
Fat mass (%)	35.7 (7.6)	139	38.2 (6.0)	95	30.2 (7.7)	44
VAT mass (g)	336.4 (177.3)	139	295.8 (162.8)	95	423.9 (177.5)	44
Trunk lean mass (kg)	20.8 (4.4)	139	18.4 (2.5)	95	25.9 (3.3)	44
Trunk fat mass (kg)	11 (4.7)	139	10.5 (4.1)	95	12 (5.7)	44
Trunk fat (%)	33 (8)	139	34.5 (7.5)	95	29.7 (8.1)	44
Arms lean mass (kg)	2.2 (0.7)	139	1.7 (0.3)	95	3.1 (0.5)	44
Arms fat mass (kg)	1.5 (0.6)	139	1.5 (0.5)	95	1.5 (0.8)	44
Arms fat (%)	38.6 (10)	139	42.6 (8)	95	29.9 (8.5)	44
Legs lean mass (kg)	6.6 (1.7)	139	5.7 (1)	95	8.6 (1.2)	44
Legs fat mass (kg)	4.9 (1.7)	139	5.1 (1.5)	95	4.6 (2)	44
Legs fat (%)	40.8 (8.7)	139	44.7 (5.2)	95	32.4 (8.7)	44
Neck measures						
Subcutaneous NAT (mL)	21.0 (27.4)	118	18.8 (15.8)	80	25.5 (42.6)	38
Intermuscular NAT (mL)	1.5 (1.6)	118	1.3 (1.1)	80	1.9 (2.3)	38
Perivertebral NAT (mL)	0.4 (0.4)	117	0.3 (0.2)	79	0.5 (0.6)	38

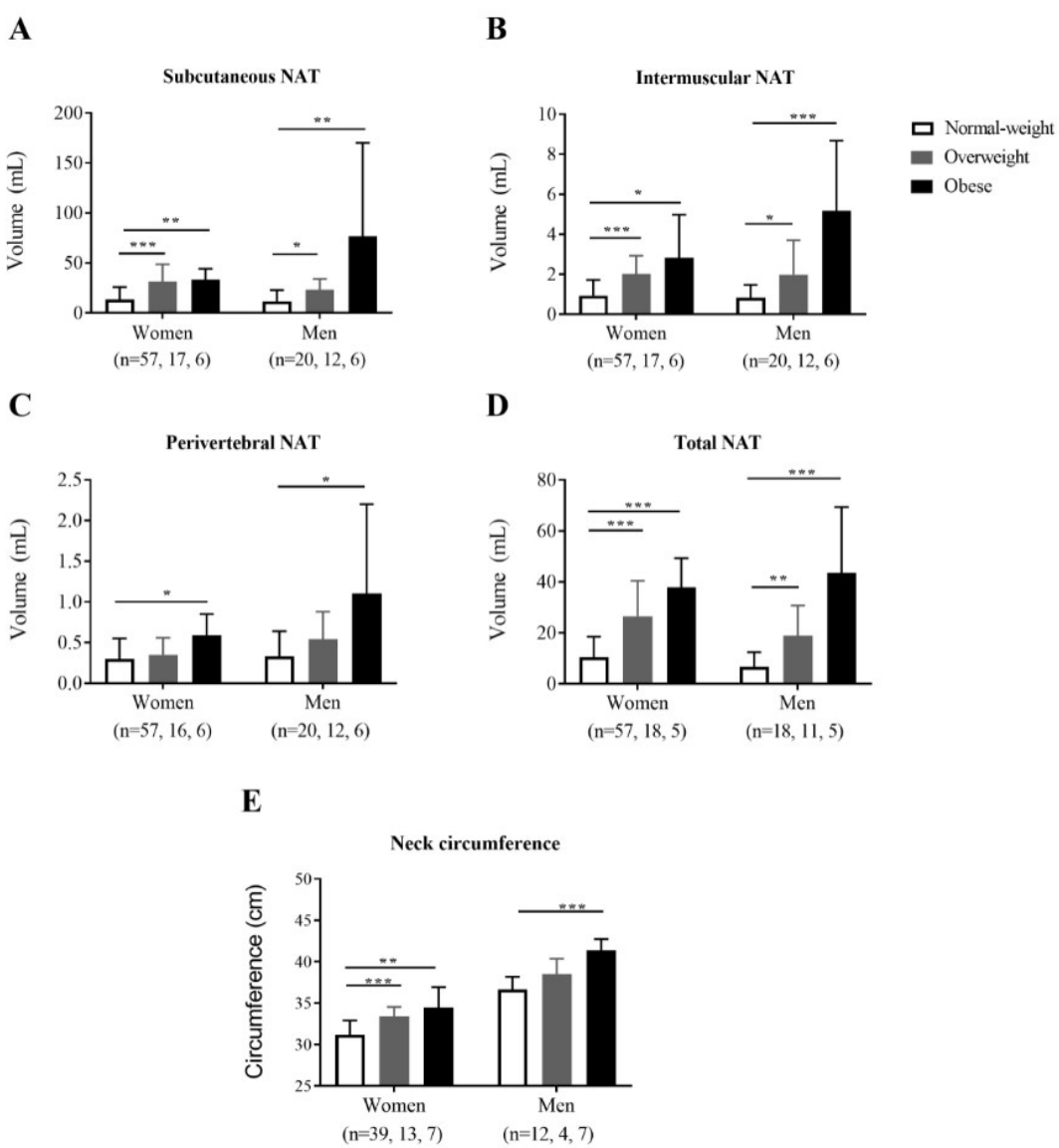
Total NAT (mL)	15.9	(14.5)	114	15.8	(13.1)	80	16.1	(17.5)	34
Neck circumference (cm)	33.8	(3.6)	82	32.1	(2.1)	59	38.4	(2.6)	23
Cardiometabolic profile									
Glucose (mg/dL)	87.7	(6.7)	137	86.9	(6.0)	93	89.4	(7.9)	44
Insulin (μIU/mL)	8.5	(5.7)	137	8.1	(4.0)	93	9.5	(8.1)	44
HOMA	1.9	(81.5)	137	1.8	(1.0)	93	2.2	(2.2)	44
TC (mg/dL)	164.8	(31.8)	137	166.3	(29.3)	93	161.7	(36.8)	44
LDL-C (mg/dL)	95.7	(26.0)	137	95.1	(24.4)	93	97.2	(29.4)	44
HDL-C (mg/dL)	52.8	(11.1)	137	56.0	(11.0)	93	46.2	(7.9)	44
TC/HDL-C	3.2	(0.9)	137	3.0	(0.6)	93	3.6	(1.1)	44
LDL-C/HDL-C	1.9	(0.7)	137	1.8	(0.6)	93	2.2	(0.8)	44
Triglycerides (mg/dL)	84.5	(51.6)	137	80.2	(46.6)	93	93.6	(60.4)	44
SBP (mmHg)	116.7	(11.9)	137	112.4	(9.5)	94	126.2	(11.2)	43
DBP (mmHg)	71.2	(7.3)	137	70.6	(6.1)	94	72.4	(9.2)	43
Muscular strength/weight	0.4	(0.1)	122	0.4	(0.1)	85	0.5	(0.1)	37
Muscular strength$_{LM}$	0.75	(0.1)	122	0.74	(0.09)	85	0.76	(0.12)	37
CRF/weight (ml/min/kg	41.3	(8.2)	133	39.5	(6.9)	94	45.5	(9.4)	39
CRF$_{LM}$ (ml/min/kg)	69.8	(10.1)	133	69.8	(9.8)	94	69.7	(11)	39
CMR-score (IDF)	0.0	(0.7)	133	-0.2	(0.5)	90	0.5	(0.8)	43
Inflammatory profile									
C-reactive protein (mg/L)	2.5	(3.5)	137	2.7	(4.0)	93	2.1	(2.3)	44
IL-2 (pg/mL)	2.4	(1.5)	114	2.7	(1.4)	76	1.9	(1.4)	38
IL-4 (pg/mL)	12.9	(9.7)	114	13.9	(9.6)	76	11	(9.7)	38
IL-6 (pg/mL)	1.7	(1.6)	114	1.7	(1.5)	76	1.6	(1.8)	38
IL-7 (pg/mL)	4	(2.8)	114	4.5	(3)	76	3.2	(2.2)	38
IL-8 (pg/mL)	1.5	(0.8)	114	1.6	(0.8)	76	1.5	(0.9)	38
IL-10 (pg/mL)	2.8	(3.5)	114	3.1	(4)	76	2.2	(2.1)	38
IL-17a (pg/mL)	4.7	(2.2)	114	5.1	(2.1)	76	4	(2.2)	38
IFNγ (pg/mL)	12.7	(5.3)	114	13.3	(5.2)	76	11.4	(5.3)	38
TNFα (pg/mL)	1.8	(1.1)	114	1.9	(1.2)	76	1.5	(0.7)	38
Complement 3 (mg/dL)	138.1	(24.1)	137	135.1	(22)	93	144.5	(27.2)	44
Complement 4 (mg/dL)	28.9	(8.9)	137	28.1	(8.1)	93	30.4	(10.2)	44
β-microglobulin 2 (mg/L)	1.3	(0.3)	137	1.3	(0.3)	93	1.3	(0.2)	44
Adiponectin (mg/L)	11.4	(7.8)	132	13.2	(8.3)	89	7.7	(5.3)	43
Leptin (μg/L)	6.1	(4.1)	134	7	(4)	91	4.3	(3.8)	43

Means (standard deviation) are provided for continuous variables and numbers (percentage) for categorical variables, together with the sample size. All values are shown without transformation. BMI: Body mass index, CMR: Cardiometabolic risk score, CRF: cardiorespiratory fitness, CRF$_{LM}$: cardiorespiratory fitness relative to lean body mass, DBP: diastolic blood pressure, HDL-C: high density lipoprotein-cholesterol, HOMA: homeostatic model assessment of insulin resistance, IDF: International Diabetes Federation; IFNγ: interferon gamma, IL: interleukin, Muscular strength$_{LM}$: muscular strength relative to lean body mass, LDL-C: low density lipoprotein-cholesterol, NAT: neck adipose tissue, SBP: systolic blood pressure, TC: total cholesterol, TNFα: tumour necrosis factor alpha, VAT: visceral adipose tissue.

NAT volume and distribution with increasing BMI

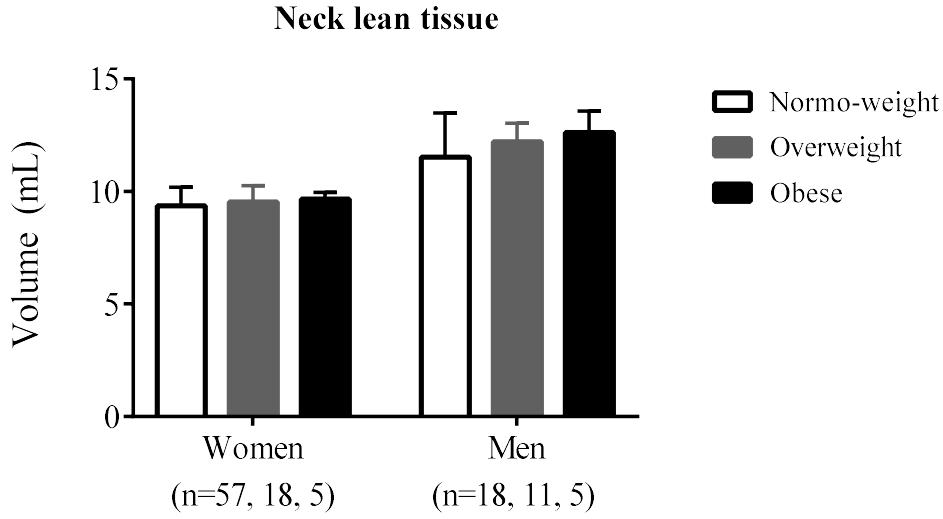
Figure 1 shows the pattern of NAT accumulation across BMI groups stratified as normal-weight, overweight and obese. The volumes of the subcutaneous, intermuscular and perivertebral and total NAT were larger in obese women and men compared to normal-weight women and men (all $P \leq 0.05$; Fig. 1 panels A, B, C and D respectively). The same pattern was seen for overweight women and men with respect to subcutaneous, intermuscular and total NAT (all $P \leq 0.05$, Panels A, B and D, respectively), but not for the perivertebral NAT ($P > 0.05$, Panel C). Unlike the NAT, neck lean tissue volume did not vary across BMI categories ($P > 0.05$, Fig. 2). Further, women who were overweight and obese, and men who were obese, returned larger NC values than their normal-weight counterparts (all $P \leq 0.05$, Panel E).

Figure 1. Mean (and standard deviation) neck measurements with respect to BMI categories. The Kruskal-Wallis test was used t
(Panels A, B and C) and total NAT volumes (Panel D), and neck circumference (Panel E), across BMI categories.



NAT variables were square root-transformed before statistical analysis to render their distribution closer to normal. *P≤0.05, **P≤0.01, ***P≤0.001. Note that subjects who were underweight were pooled with the normal-weight subject. Panel F shows the NAT compartments in a typical normal-weight, overweight and obese subject.

Figure 2. Mean (standard deviation) neck lean tissue volumes with respect to BMI categories.

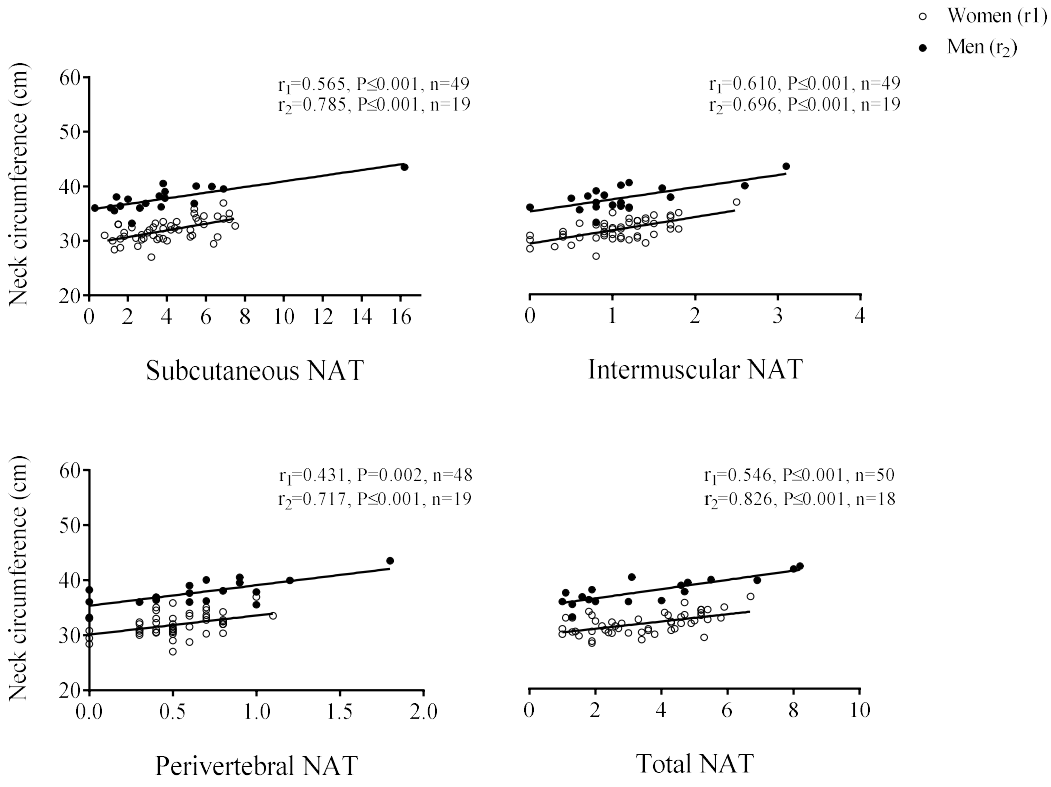


The Kruskal-Wallis test was used to compare the results for the BMI categories. Total neck lean mass values were square root-transformed to render their distribution closer to normal. P≤0.05, **P≤0.01, ***P≤0.001). Note: subjects who were underweight were pooled with the normal-weight subjects.

Associations of NAT volume and its distribution with anthropometric and body compositions parameters

The volume of subcutaneous, intermuscular, perivertebral and total NAT was directly associated with NC in both women and men (all P≤0.002, Fig. 3), although these correlations were weaker in women than in men (r=0.43-0.61 vs. 0.70-0.83, respectively).

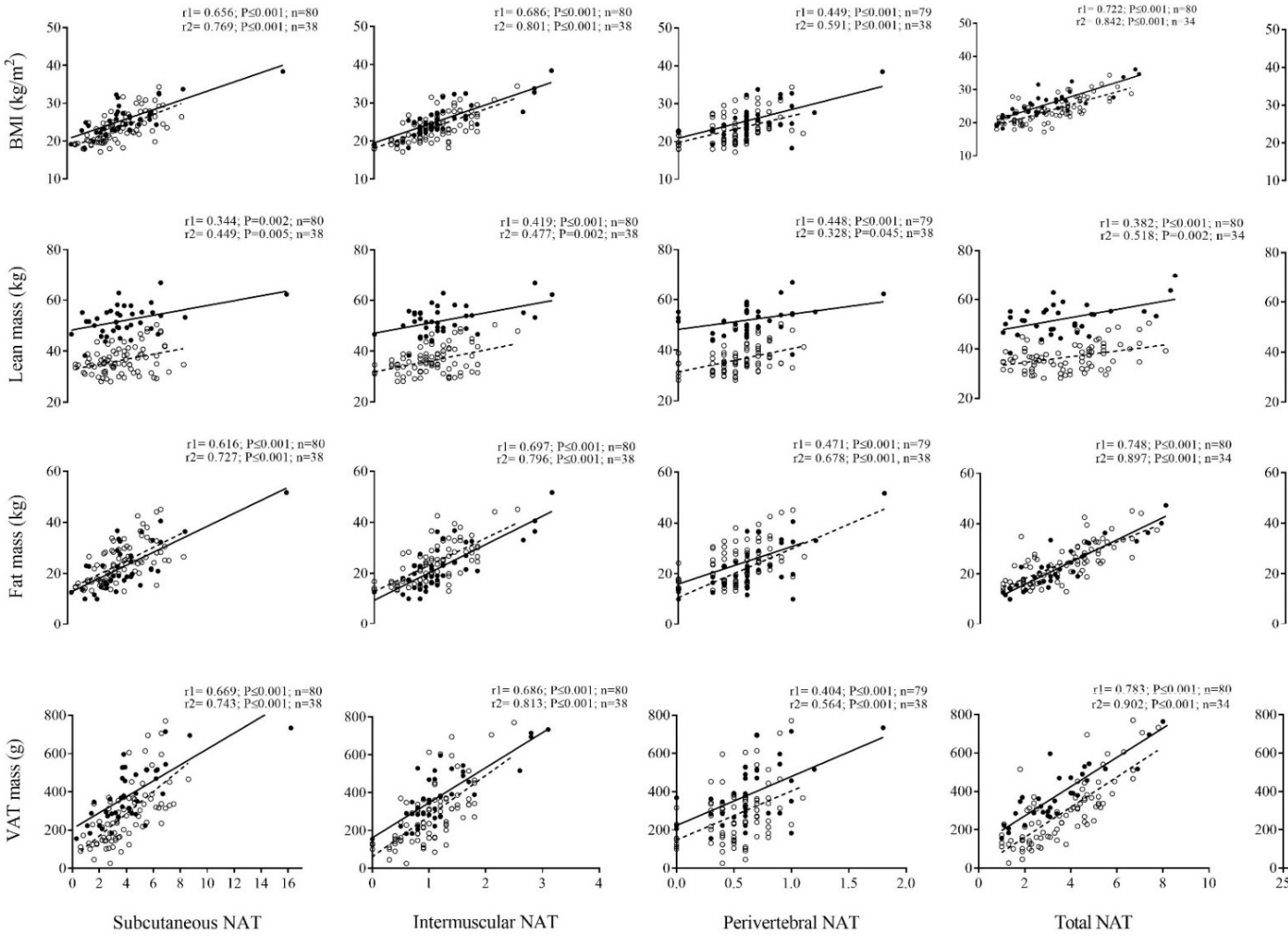
Figure 3. Association between neck adipose tissue (NAT) and neck circumference with respect to sex.



Pearson correlation coefficients, P-values and sample sizes are provided for both women and men. All NAT-related variables were square root-transformed to render distribution closer to normal.

The different compartmental and total NAT volumes were also directly associated with BMI, lean mass, fat mass, and VAT mass, in both sexes (all P≤0.05, Fig. 4). The intermuscular and total NAT volumes showed the strongest correlations with BMI, fat mass and VAT mass, with r>0.69 in women, and >0.8 in men. The NC also showed direct relationships with the same variables.

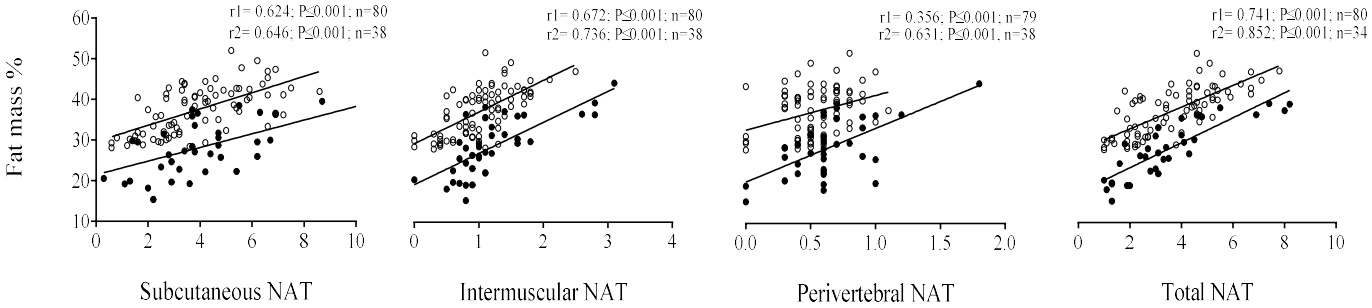
Figure 4. Association between neck measurements and body composition in both sexes.



Pearson correlation coefficients, P-values and sample sizes are provided for both women and men. NAT variables were square
analysis to render their distribution closer to normal. After applying False Discovery Rate (FDR, Benjamini-Hochberg) correctio
statistically significant (P≤0.05). NAT: Neck adipose tissue; VAT: Visceral adipose tissue.

These results remained similar when fat mass was substituted by percentage fat mas, although the strength
slightly less strong (see Fig. 5).

Figure 5. Association between neck measurements and percentage fat mass with respect to sex.



Pearson correlation coefficients, P-values and sample sizes are provided for women and men. All NAT-related variables were sq
their distribution closer to normal.

Neck lean tissue volume was only associated with overall lean mass in women (P≤0.001, Table 2).

Table 2. Association between the neck lean tissue volume at the height of C5 with other neck measurements, anthropometric results, body composition variables, cardiometabolic risk, and inflammatory profile.

	WOMEN		**MEN**	
	r	**n**	**r**	**n**
Neck measurements				
Subcutaneous NAT	0.08	(93)	0.02	(43)
Intermuscular NAT	0.13	(93)	0.02	(43)
Perivertebral NAT	0.12	(92)	0.14	(43)
Total NAT	0.04	(89)	0.02	(34)
Neck circumference (cm)	**0.58*** **	(50)	**0.53***	(24)
Anthropometry and body composition				
BMI (kg/m^2)	0.16	(80)	0.15	(34)
Lean mass (kg)	**0.49*** **	(80)	0.31	(34)
Fat mass (kg)	0.13	(80)	0.12	(34)
Body fat percentage (%)	-0.10	(80)	0.004	(34)
VAT mass (g)	0.05	(80)	0.16	(34)
Cardiometabolic profile				
Glucose	0.06	(78)	-0.07	(34)
Insulin	0.06	(78)	-0.03	(34)
HOMA	0.07	(78)	-0.03	(34)
TC	-0.09	(78)	-0.17	(34)
LDL-C	-0.01	(78)	-0.15	(34)
HDL-C	-0.07	(78)	-0.11	(34)
TC/HDL-C	-0.01	(78)	-0.05	(34)
LDL-C/HDL-C	0.04	(78)	-0.05	(34)
Triglycerides	-0.21	(78)	-0.13	(34)
SBP	**0.23***	(79)	-0.05	(33)
DBP	0.01	(79)	-0.01	(33)
Muscular strength/weight	-0.05	(73)	-0.06	(28)
Muscular strength$_{LM}$	-0.14	(73)	0.09	(28)
CRF/weight (ml/min/kg)	-0.01	(79)	0.33	(29)
CRF$_{LM}$	-0.12	(79)	**0.38***	(29)
CMR	0.08	(75)	-0.03	(34)
Inflammatory profile				
C-reactive protein	-0.06	(78)	0.06	(34)
IL-2	-0.04	(63)	-0.28	(30)
IL-4	-0.14	(63)	**-0.37***	(30)
IL-6	-0.02	(63)	-0.21	(30)
IL-7	-0.14	(63)	**-0.40***	(30)
IL-8	-0.04	(63)	-0.36	(30)
IL-10	0.18	(63)	-0.25	(30)
IL-17a	0.14	(63)	**-0.43***	(30)
IFNɣ	**0.30***	(63)	-0.25	(30)
TNFα	**0.26***	(63)	-0.35	(30)
Complement 3	0.04	(78)	-0.11	(34)
Complement 4	-0.09	(78)	0.08	(34)
β-microglobulin 2	0.10	(78)	-0.01	(34)
Adiponectin	0.02	(74)	0.08	(33)
Leptin	-0.01	(76)	-0.18	(33)

Bivariate correlations were performed to examine all associations. Pearson correlation coefficients and sample sizes are provided. Significant differences are indicated by asterisks: *P≤0.05, **P≤0.01, ***P≤0.001. All variables related to neck lean tissue volume, cardiometabolic profile (except muscular and cardiorespiratory fitness), and inflammatory markers were square root-transformed to render their distribution closer to normal. CMR: Cardiometabolic risk score, CRF: cardiorespiratory fitness, CRF_{LM}: cardiorespiratory fitness relative to lean body mass, DBP: diastolic blood pressure, HDL: high density lipoprotein-cholesterol, HOMA: homeostatic model assessment of insulin resistance, IFNγ: interferon gamma, IL: interleukin, LDL-C: low density lipoprotein-cholesterol, $Muscular\ strength_{LM}$: muscular strength relative to lean body mass, TC: total cholesterol, SBP: systolic blood pressure, TNFα: tumour necrosis factor-alpha, VAT: Visceral adipose tissue.

The table 3, show the relationship of compartmental and total NAT with trunk and appendicular lean mass, and fat mass and percentage.

Table 3. Associations between neck measurements with trunk and appendicular lean mass, and fat mass and percentage.

	Subcutaneous NAT				**Intermuscular NAT**				**Perivertebral NAT**				**Total NAT**			
	Women		Men		Women		Men		Women		Men		Women		Me	
	r	n	r	n	r	n	r	n	r	n	r	n	r	n	r	
Trunk fat mass	**0.66*****	(80)	**0.74*****	(38)	**0.72*****	(80)	**0.81*****	(38)	**0.45*****	(79)	**0.69*****	(38)	**0.81*****	(80)	**0.90*****	
Trunk fat (%)	**0.68*****	(80)	**0.66*****	(38)	**0.72*****	(80)	**0.76*****	(38)	**0.38*****	(79)	**0.65*****	(38)	**0.82*****	(80)	**0.87*****	
Trunk lean mass	**0.3****	(80)	**0.52*****	(38)	**0.36*****	(80)	**0.50*****	(38)	**0.37*****	(79)	**0.39***	(38)	**0.32****	(80)	**0.55*****	
Arm fat mass	**0.61*****	(80)	**0.83*****	(38)	**0.69*****	(80)	**0.84*****	(38)	**0.47*****	(79)	**0.70*****	(38)	**0.77*****	(80)	**0.91*****	
Arms fat (%)	**0.59*****	(80)	**0.70*****	(38)	**0.62*****	(80)	**0.76*****	(38)	**0.36*****	(79)	**0.63*****	(38)	**0.72*****	(80)	**0.84*****	
Arms lean mass	**0.28***	(80)	0.31	(38)	**0.32****	(80)	0.29	(38)	**0.34****	(79)	**0.19**	(38)	**0.30****	(80)	**0.39***	
Legs fat mass	**0.49*****	(80)	**0.63*****	(38)	**0.58*****	(80)	**0.72*****	(38)	**0.44*****	(79)	**0.61*****	(38)	**0.56*****	(80)	**0.84*****	
Legs fat (%)	**0.42*****	(80)	**0.55*****	(38)	**0.46*****	(80)	**0.64*****	(38)	0.21	(79)	**0.55*****	(38)	**0.44*****	(80)	**0.77*****	
Legs lean mass	**0.38*****	(80)	0.31	(38)	**0.48*****	(80)	**0.38***	(38)	**0.51*****	(79)	0.21	(38)	**0.44*****	(80)	**0.40***	

Bivariate correlations were performed to examine all associations. Pearson correlation coefficients and sample sizes are provide
indicated by asterisks: *P≤0.05, **P≤0.01, ***P≤0.001. All neck adipose tissue (NAT) measures were square root-transformed to re
to normal.

Association of NAT volume and its distribution with cardiometabolic risk and inflammatory profile

Larger NAT accumulations were associated with higher CMR scores in both women and men. The compartmental and (especially) total NAT volumes, and the NC, were directly associated with glycaemic and lipid markers and blood pressure (all CMR factors), and inversely with HDL-C and some physical fitness components (all $P \leq 0.05$, see Table 4). These associations appeared to be stronger in the men than in the women. Total NAT volume and VAT mass were equally related to the CMR score (for both associations $r=0.6$ and 0.8 for women and men respectively).

Table 4. Association between neck measurements/visceral adipose tissue (VAT) and cardiometabolic risk (CMR) factors in wom

	Subcutaneous NAT				**Intermuscular NAT**				**Perivertebral NAT**				**Total NAT**				**Neck circumfe**	
	Women		Men		Women		Men		Women		Men		Women		Men		Women	
	r	n	r	n	r	n	r	n	r	n	r	n	r	n	r	n	r	n
Glucose	0.18	(78)	0.29	(38)	0.22	(78)	**0.30**	(38)	0.20	(77)	0.21	(38)	**0.25***	(78)	**0.63*****	(34)	**0.32***	(58)
Insulin	**0.44*****	(78)	**0.50*****	(38)	**0.41*****	(78)	**0.63*****	(38)	**0.28***	(77)	**0.49****	(38)	**0.59*****	(78)	**0.68*****	(34)	**0.54*****	(58)
HOMA	**0.43*****	(78)	**0.52*****	(38)	**0.41*****	(78)	**0.65*****	(38)	**0.29***	(77)	**0.49****	(38)	**0.57*****	(78)	**0.69*****	(34)	**0.55*****	(58)
Total cholesterol	-0.07	(78)	0.19	(38)	0.02	(78)	0.34*	(38)	0.07	(77)	0.18	(38)	-0.13	(78)	**0.46****	(34)	0.12	(58)
LDL-C	-0.01	(78)	0.23	(38)	0.13	(78)	**0.37***	(38)	0.20	(77)	0.22	(38)	-0.04	(78)	**0.48****	(34)	0.21	(58)
HDL-C	**-0.30****	(78)	-0.16	(38)	**-0.37*****	(78)	-0.35*	(38)	**-0.31****	(77)	-0.18	(38)	**-0.39*****	(78)	**-0.53*****	(34)	-0.20	(58)
TC/HDL-C	0.24*	(78)	0.27	(38)	**0.39*****	(78)	**0.53****	(38)	**0.36*****	(77)	0.27	(38)	**0.27***	(78)	**0.73*****	(34)	**0.32***	(58)
LDL-C/HDL-C	0.19	(78)	0.28	(38)	**0.34****	(78)	**0.50****	(38)	**0.35****	(77)	0.27	(38)	0.21	(78)	**0.68*****	(34)	**0.32***	(58)
Triglycerides	**0 .25***	(78)	0.14	(38)	**0.37*****	(78)	**0.39***	(38)	0.18	(77)	0.23	(38)	**0.27***	(78)	**0.63*****	(34)	0.23	(58)
SBP	**0.31****	(79)	0	(37)	**0.38*****	(79)	0.07	(37)	**0.37*****	(78)	-0.13	(37)	**0.33****	(79)	0.28	(33)	**0.47*****	(58)
DBP	0.24*	(79)	0.10	(37)	0.22*	(79)	0.23	(37)	0.12	(78)	0.04	(37)	**0.29****	(79)	**0.38***	(33)	0.14	(58)
Muscular strength/weight	**-0.53 *****	(72)	**-0.52****	(31)	**-0.62*****	(72)	**-0.68*****	(31)	**-0.38*****	(71)	-0.35	(31)	**-0.59*****	(73)	**-0.65*****	(28)	**-0.39****	(59)
Muscular strength$_{LM}$	**-0.33****	(72)	-0.28	(31)	**-0.41*****	(72)	**-0.45***	(31)	**-0.28***	(71)	-0.07	(31)	**-0.36****	(73)	**-0.31**	(28)	-0.26*	(59)
CRF/weight	**-0.41*****	(79)	**-0.45****	(34)	**-0.36*****	(79)	**-0.55*****	(34)	-0.07	(78)	**-0.56*****	(34)	**-0.57*****	(79)	**-0.43***	(29)	**-0.41*****	(59)
CRF$_{LM}$	-0.08	(79)	-0.18	(34)	-0.002	(79)	-0.23	(34)	0.11	(78)	-0.28	(34)	-0.18	(79)	0.03	(29)	-0.22	(59)
CMR score	**0.55*****	(76)	**0.49****	(37)	**0.66*****	(76)	**0.64*****	(37)	**0.45*****	(75)	**0.37***	(37)	**0.62*****	(75)	**0.82*****	(33)	**0.61*****	(57)

Pearson correlation coefficients were determined to examine the association of neck adipose tissue (NAT) and circumference, and VAT m markers, blood pressure and physical fitness. *P≤0.05, **P≤0.01, ***P≤0.001. Values that remained statistically significant (P≤0.05) after applyi (Benjamini-Hochberg) are shown in bold. Of note, we followed a conservative approach, pooling all comparisons together, by sex. All NAT an (except for muscular and cardiorespiratory fitness) were square root-transformed to bring their distributions closer to normal. CR cardiorespiratory fitness relative to lean body mass, DBP: diastolic blood pressure, HDL: high-density lipoprotein-cholesterol, HOMA: homeo resistance, LDL-C: low-density lipoprotein-cholesterol, LDL-C/HDL-C: low-density lipoprotein cholesterol (LDL-C)/HDL-C ratio, TC/HDL-C: lipoprotein cholesterol (HDL-C) ratio, SBP: systolic blood pressure, VAT: visceral adipose tissue.

Table 5 shows that, in women, the compartmental and total NAT volumes were directly associated with the concentrations of C3 and C4 (except for the perivertebral NAT), and leptin, and inversely associated with the adiponectin concentration (all $P \leq 0.01$). In men, the compartmental and total NAT volumes were directly associated with the C3 and leptin concentrations (both $P \leq 0.001$), and inversely associated with that of adiponectin ($P \leq 0.05$, except the perivertebral NAT). Perivertebral NAT was the only NAT compartment volume associated with the C4 concentration ($P \leq 0.01$). The NC was directly associated with the C4 and leptin concentrations in both women and men (all $P \leq 0.01$). It is noteworthy that, overall, subcutaneous, intermuscular and total NAT, and VAT mass, were similarly associated with the C3 ($r=0.41$ to 0.50 in women, $r=0.59$ to 0.74 in men), C4 ($r=0.32$ to 0.39 in women), adiponectin ($r=-0.24$ to -0.31 in women; $r=-0.43$ to -0.56 in men) and leptin concentrations ($r=0.38$ to 0.59 in women, $r=0.57$ to 0.69 in men).

Table 5. Association between neck measurements/visceral adipose tissue (VAT) mass and inflammatory markers in women and

	Subcutaneous NAT				**Intermuscular NAT**				**Perivertebral NAT**				**Total NAT**				**Neck circum**	
	Women		Men		Women		Men		Women		Men		Women		Men		Women	
	r	n	r	n	r	n	r	n	r	n	r	n	r	n	r	n	r	n
C-reactive protein	**0.29***	(78)	0.21	(38)	**0.377*****	(78)	0.20	(38)	0.17	(78)	**0.52*****	(38)	**0.39*****	(78)	0.19	(34)	0.12	(58)
IL-2	-0.19	(64)	0.13	(34)	-0.17	(64)	-0.11	(34)	-0.05	(64)	-0.13	(34)	-0.06	(63)	-0.09	(30)	0.08	(42)
IL-4	-0.14	(64)	0.24	(34)	-0.12	(64)	-0.01	(34)	0.02	(64)	-0.17	(34)	-0.06	(63)	-0.05	(30)	0.13	(42)
IL-6	-0.13	(64)	0.20	(34)	-0.06	(64)	0.00	(34)	0.03	(64)	0.01	(34)	0.05	(63)	-0.05	(30)	0.17	(42)
IL-7	**-0.32****	(64)	0.28	(34)	-0.29*	(64)	0.04	(34)	-0.08	(64)	-0.26	(34)	-0.23	(63)	-0.04	(30)	-0.15	(42)
IL-8	-0.07	(64)	0.32	(34)	0.02	(64)	0.10	(34)	-0.003	(64)	0.05	(34)	0.07	(63)	0.17	(30)	0.02	(42)
IL-10	-0.04	(64)	0.003	(34)	0.02	(64)	-0.43	(34)	0.08	(64)	-0.40*	(34)	-0.03	(63)	0.10	(30)	0.04	(42)
IL-17a	-0.05	(64)	0.19	(34)	0.06	(64)	0.05	(34)	0.03	(64)	-0.19	(34)	-0.09	(63)	0.12	(30)	-0.01	(42)
IFNɣ	0.07	(64)	-0.13	(34)	0.11	(64)	-0.15	(34)	-0.06	(64)	-0.18	(34)	0.02	(63)	-0.01	(30)	-0.03	(42)
TNFα	0.04	(64)	0.03	(34)	0.14	(64)	-0.07	(34)	-0.11	(64)	-0.13	(34)	0.08	(63)	0.05	(30)	0.19	(42)
Complement 3	**0.41*****	(78)	**0.59*****	(38)	**0.48*****	(78)	**0.60*****	(38)	0.21	(78)	**0.58*****	(38)	**0.46*****	(78)	**0.62*****	(34)	**0.40****	(58)
Complement 4	**0.32****	(78)	0.18	(38)	**0.32****	(78)	0.28	(38)	0.14	(78)	**0.43****	(38)	**0.39*****	(78)	0.31	(34)	0.21	(58)
β-microglobulin 2	0.18	(78)	-0.31	(38)	**0.28***	(78)	-0.36*	(38)	0.13	(78)	-0.12	(38)	0.19	(78)	0.01	(34)	-0.27*	(58)
Adiponectin	**-0.31****	(75)	**-0.43****	(37)	**-0.31****	(75)	**-0.52*****	(37)	**-0.33****	(75)	-0.32	(37)	**-0.31****	(74)	**-0.56*****	(33)	-0.15	(54)
Leptin	**0.38*****	(76)	**0.57*****	(37)	**0.46*****	(76)	**0.67*****	(37)	**0.37*****	(76)	**0.63*****	(37)	**0.59*****	(76)	**0.69*****	(33)	**0.37****	(54)

Pearson correlation coefficients were determined to examine the association between neck measurements and inflammato
***P≤0.001. Values that remained statistically significant (P≤0.05) after applying False Discovery Rate correction (Benjamini-Ho
note, we followed a conservative approach, pooling all comparisons together, by sex. All NAT and cardiometabolic profil
transformed to bring their distributions closer to normal. IL: interleukin, IFNɣ: interferon gamma, TNFα: tumour necrosis facto
tissue.

After adjusting for multiple comparisons, most results related to the main analyses (see Figure 4, Tables 4 and 5), remained similar. Of note, some of these relationships (for instance that of intermuscular and total NAT with the CMR score) remained significant (P≤0.05) in women or/and men, independently of BMI, body fat percentage and VAT mass (Table S1, Supporting Information) (31). Furthermore, we found that when examining the relationship with cardiometabolic markers, total NAT and VAT mass outperformed BMI and body composition parameters in most associations in men. Similarly, total and intermuscular NAT, and neck circumference generally were more strongly associated with glycaemic and lipid markers than BMI and body composition parameters. Regarding the relationship with inflammatory markers in men, total and intermuscular NAT did not outperform BMI and body composition parameters. However, in women, intermuscular and total NAT were more strongly associated with C-reactive protein, several components of the natural immune system and adiponectin than the rest of anthropometric and body composition parameters (see Tables S2 and S3 of the Supporting Information) (31).

Sensitivity analyses are provided in the Supporting Information (31). Coefficients of variation (CVs) of NAT measures (indicating the consistency of our data), and Bland-Altman plots comparing inter-evaluator estimate differences in NAT assessment, are also provided in the Supporting Information (31).

DISCUSSION

The present results show NAT accumulation to be greater in those subjects with higher adiposity values, and that the compartmental and total NAT volumes are associated with overall and central adiposity. The compartmental (especially intermuscular) and total NAT volumes were also directly associated with CMR and inflammatory status. Several of these associations remained statistically significant independently of BMI, body fat percentage and VAT mass, such as is the case for the relationship between total NAT volume and the CMR score. These findings suggest that total NAT volume might be as valuable as VAT mass in terms of predicting CMR and inflammatory status, and suggest that NC might be a useful clinical variable for estimating CMR, especially in men.

Torriani et al. (9) recently reported that the NAT increases with increasing adiposity, and that it seems to follow different accumulation patterns across the subcutaneous, intermuscular and perivertebral compartments, with each differently related to CMR, in patients with successfully treated malignant/benign tumours. The present results are in line with these findings, although the perivertebral NAT seemed to be less affected by increasing adiposity. Indeed, the compartmental NAT volumes were similarly related to total and central body composition variables, but the perivertebral NAT volume returned the weakest associations. Also in agreement with the above authors, the intermuscular and total NAT volumes showed the strongest association with the CMR factors examined, and total NAT seemed to be as predictive as VAT mass with respect to overall CMR. Interestingly, potential differences between the sexes were seen in the relationship between the NAT variables and body composition and CMR, which were stronger in men. It is known that women are more likely to accumulate fat in the lower body (i.e., the gluteofemoral zone) than are men, who tend to accumulate more visceral fat (33), and that this body fat distribution is differently related to CMR (34). Thus, it might be speculated

that NAT accumulation is also different in women and men and might also be differently related to CMR. Torriani et al. (9) previously showed that women were more likely to accumulate neck fat in the subcutaneous NAT compartment, and men in the intermuscular and perivertebral NAT compartments. This finding, together with the present results, reinforces the hypothesis that NAT accumulation in specific compartments is gender-dependent, and that it might be differently related to CMR (more so in men). However, this might be partially driven by the fact that men had a higher average BMI in the present cohort. Together, these findings underline the relationship between NAT accumulation and cardiometabolic disease, and shed some light on traditionally non-explored adipose fat deposits that might provide therapeutic targets.

To better understand the pathophysiology of NAT (and its specific distribution), studies are required that examine whether NAT accumulation is related to the low grade pro-inflammatory status commonly associated with obesity (35). The present work therefore examined whether the compartmental and total NAT volumes were associated with the systemic anti- and pro-inflammatory factors previously shown to be associated with VAT (20). Overall, the present results show the intermuscular and total NAT volumes and the VAT mass to be similarly related, in terms of direction and strength, to the C3, C4, adiponectin and leptin concentrations. The lack of studies in this area precludes comparisons being made, although some (36; 37) have compared inflammation signalling in gluteal and abdominal subcutaneous white adipose tissue, and report the relationship between the expression of inflammatory or cytokine genes in the former region in persons with obesity comorbidities (e.g., hyperlipidaemia and insulin resistance) to be considerably weaker than that seen in the latter. This suggests that the accumulation of fat - including the NAT - across specific adipose tissue deposits, might contribute differently to the low-grade chronic pro-inflammatory state. This idea warrants further research.

From a clinical point of view, the volumetric quantification of NAT might not be viable given the high cost, technical difficulties and exposure to radiation involved in PET/CT imaging. However, the NC showed correlation coefficients with CMR factors (Table 1) similar to those of the compartmental and total NAT volumes. In addition, the NC showed strong and moderate-to-strong associations with the NAT volumes in both men and women. These findings are in line with those of large cohort studies showing NC to be directly associated with a large battery of CMR markers (38-40), and moderately associated with upper body subcutaneous fat volume (35). Although NC did not show such a strong relationship with systemic inflammatory markers as did the intermuscular and total NAT volumes, it would seem to be a good marker of CMR in young, healthy, sedentary adults, and might provide a practical screening tool for determining the latter.

These results should be interpreted with caution. They may not be generalizable to people with excess upper body fat due to the difficulties of accurately outlining the ROIs for distinguishing specific NAT compartments. A multidetector-based analysis of a neck area beyond that studied in the present work, as described elsewhere (10), may help better characterize the NAT. Further, since a thin pillow was placed below 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. In addition, the subjects underwent a personalized cold exposure prior to their PET/CT scan, which might have had a very small effect (cold exposure only induces a mean change of only ~3 HU) (41) on the radiodensity readings, leading to a small number of voxels that should have been classified as NAT. Of note, CMR and inflammation parameters were normally assessed within 3 weeks of the 18F-FDG-PET/CT assessment. Further work should examine the molecular signatures of the neck region to try to reveal the

underlying mechanisms (e.g., lipid metabolism and regulation) by which NAT accumulation contributes to a higher CMR and a more pro-inflammatory status.

In conclusion, an increase in NAT volume is associated with a higher CMR and a more pro-inflammatory state in young, healthy, sedentary adults. Some of the relationships of NAT measures with CMR and inflammatory markers were independent of BMI, body fat percentage and/or VAT mass. Our findings suggest that total NAT volume might be as valuable as VAT mass in terms of predicting CMR and inflammatory status, and suggest that NC might be a useful clinical variable for estimating CMR, especially in men. Further research is warranted to understand the mechanisms giving rise to these associations.

SUPPORTING INFORMATION

Table S1. Relationship of neck measures with body composition, the cardiometabolic and inflammatory profile after adjusting f
(body mass index, body fat percentage, and VAT mass), in women and men.

					WOMEN			
		Subcutaneous NAT	**Intermuscular NAT**	**Perivertebral NAT**		**Total NAT**	**Total NAT/height**	**NAT pe (%)**
Model 1 (adjusted for BMI)								
	n	r	r	R	n	r	r	
Body composition								
BMI (Kg/m²)								
Lean mass (kg)	80	-0.20	-0.10	0.21	80	-0.21	**-0.33****	**-0.34****
Fat mass (kg)	80	0.04	**0.23***	0.17	80	**0.31****	0.21	**0.29****
VAT mass (g	80	**0.28***	**0.27***	0.05	80	**0.48*****	**0.44*****	**0.46*****
Cardiometabolic profile								
Glucose	78	0.00	0.05	0.09	78	0.11	0.10	0.08
Insulin	78	0.18	0.12	0.08	78	**0.41*****	**0.41*****	**0.36*****
HOMA	78	0.17	0.12	0.08	78	**0.40*****	**0.39*****	**0.34****
Total cholesterol	78	0.02	0.15	0.14	78	-0.02	-0.03	0.02
LDL-C	78	-0.01	0.17	0.22	78	-0.05	-0.06	-0.03
HDL-C	78	-0.05	-0.14	-0.15	78	-0.14	-0.16	-0.12
TC/HDL-C	78	0.05	**0.26***	**0.26***	78	0.10	0.11	0.12
LDL-C/HDL-C	78	0.01	0.22	**0.27***	78	0.03	0.03	0.03
Triglycerides	78	0.14	**0.31****	0.10	78	0.22	**0.23***	**0.26***
SBP	79	-0.02	0.07	0.19	79	-0.01	-0.04	-0.05
DBP	79	0.06	0.04	-0.02	79	0.11	0.11	0.09
Muscular strength/weight	72	-0.09	-0.22	-0.07	73	-0.12	-0.12	-0.16
CRF/weight	79	-0.11	-0.01	0.21	79	**-0.31****	**-0.29***	**-0.32****
CMR score	76	0.16	**0.34****	0.22	75	**0.26***	**0.26***	**0.23***
Inflammatory profile								
C-reactive protein	78	0.07	0.19	0.01	78	0.13	0.12	0.15

IL-2	64	-0.12	-0.09	0.02	63	-0.10	-0.08	-0.09
IL-4	64	-0.15	-0.12	0.04	63	-0.14	-0.12	-0.09
IL-6	64	-0.15	-0.06	0.05	63	-0.03	-0.01	-0.03
IL-7	64	-0.24	-0.18	0.03	63	-0.15	-0.14	-0.13
IL-8	64	-0.07	0.05	0.01	63	0.02	0.04	0.02
IL-10	64	-0.07	0.00	0.08	63	-0.20	-0.19	-0.23
IL-17a	64	0.03	0.20	0.09	63	-0.16	-0.17	-0.19
IFNɣ	64	0.07	0.13	-0.08	63	-0.13	-0.12	-0.19
TNFα	64	-0.02	0.11	-0.17	63	-0.12	-0.11	-0.17
Complement 3	78	0.21	**0.30****	0.03	78	**0.24***	0.22	**0.24***
Complement 4	78	0.18	0.17	0.01	78	**0.27***	**0.28***	**0.29***
β-microglobulin 2	78	-0.05	0.09	-0.02	78	-0.04	-0.04	-0.05
Adiponectin	75	**-0.24***	**-0.24***	**-0.27***	74	**-0.29***	**-0.29***	**-0.29***
Leptin	76	0.00	0.11	0.15	76	**0.28***	**0.24***	**0.28***
Model 2 (adjusted for body fat percentage)								
Body composition								
BMI (kg/m$^{2)}$	80	**0.35****	**0.35****	**0.29****	80	**0.33****	**0.31****	0.21
Lean mass (kg)	80	**0.23***	**0.33****	**0.39*****	80	**0.29***	0.18	0.12
Fat mass (kg)								
VAT mass (g)	80	**0.37*****	**0.35****	0.22	80	**0.48*****	**0.44*****	**0.40*****
Cardiometabolic profile								
Glucose	78	0.09	0.14	0.15	78	0.20	0.18	0.16
Insulin	78	**0.25***	0.19	0.15	78	**0.48*****	**0.48*****	**0.42*****
HOMA	78	**0.25***	0.20	0.16	78	**0.48*****	**0.47*****	**0.41*****
Total cholesterol	78	-0.05	0.08	0.09	78	-0.08	-0.08	-0.02
LDL-C	78	-0.01	0.17	-0.21	78	-0.04	-0.05	-0.02
HDL-C	78	-0.12	-0.21	0.21	78	-0.17	-0.19	-0.13
TC/HDL-C	78	0.08	**0.27***	**0.29***	78	0.10	0.10	0.10
LDL-C/HDL-C	78	0.07	**0.27***	**0.30****	78	0.07	0.07	0.06
Triglycerides	78	0.07	**0.23***	0.08	78	0.13	0.15	0.19
SBP	79	0.13	0.22	**0.28***	79	0.15	0.12	0.07
DBP	79	0.12	0.09	0.04	79	0.17	0.16	0.13

Muscular strength/weight	72	-0.12	-0.22	-0.17	73	-0.05	-0.03	0.00
CRF/weight	79	-0.09	0.02	0.16	79	**-0.27***	**-0.24***	**-0.25***
CMR score	76	**0.31****	**0.46*****	**0.33****	75	**0.39*****	**0.38*****	**0.33****
Inflammatory profile								
C-reactive protein	78	0.10	0.21	0.06	78	0.13	0.11	0.12
IL-2	64	-0.10	-0.08	0.01	63	-0.06	-0.04	-0.06
IL-4	64	-0.14	-0.12	0.04	63	-0.16	-0.14	-0.13
IL-6	64	-0.15	-0.06	0.04	63	-0.03	-0.01	-0.04
IL-7	64	**-0.26***	-0.21	-0.01	63	-0.16	-0.15	-0.13
IL-8	64	-0.16	-0.05	-0.04	63	-0.09	-0.07	-0.10
IL-10	64	-0.03	0.04	0.09	63	-0.17	-0.17	-0.23
IL-17a	64	0.02	0.18	0.07	63	-0.19	-0.20	-0.24
IFNɣ	64	0.15	0.22	-0.04	63	0.00	0.00	-0.09
TNFα	64	0.02	0.15	-0.14	63	-0.05	-0.04	-0.13
Complement 3	78	0.21	**0.29***	0.07	78	0.22	0.20	0.19
Complement 4	78	0.13	0.10	0.01	78	0.19	0.20	0.20
β-microglobulin 2	78	0.04	0.17	0.04	78	0.03	0.03	0.00
Adiponectin	75	-0.22	-0.21	**-0.27***	74	**-0.25***	**-0.26***	**-0.26***
Leptin	76	0.01	0.10	0.21	76	**0.23***	0.19	0.19
Model 3 (adjusted for VAT mass)								
Body composition								
BMI (kg/m$^{2)}$	80	**0.23***	**0.27***	**0.22***	80	0.21	0.22	0.15
Lean mass (kg)	80	-0.08	0.03	**0.29***	80	-0.07	-0.18	**-0.23***
Fat mass (kg)	80	0.07	**0.27***	**0.27***	80	0.22	0.15	0.17
VAT mass (g)								
Cardiometabolic profile								
Glucose	78	0.00	0.06	0.11	78	0.10	0.09	0.07
Insulin	78	0.16	0.11	0.10	78	**0.40*****	**0.40*****	**0.34****
HOMA	78	0.16	0.12	0.11	78	**0.39*****	**0.38*****	**0.32****
Total cholesterol	78	-0.04	0.08	0.10	78	-0.10	-0.11	-0.05
LDL-C	78	-0.02	0.16	0.21	78	-0.06	-0.06	-0.03
HDL-C	78	-0.11	-0.21	-0.20	78	**-0.24***	**-0.25***	-0.20

TC/HDL-C	78	0.05	**0.26***	**0.27***	78	0.12	0.13	0.13
LDL-C/HDL-C	78	0.04	**0.25***	**0.29***	78	0.08	0.08	0.08
Triglycerides	78	0.01	0.18	0.05	78	0.06	0.08	0.12
SBP	79	-0.06	0.04	0.20	79	-0.06	-0.08	-0.12
DBP	79	-0.01	-0.04	-0.03	79	-0.01	0.00	-0.03
Muscular strength/weight	72	-0.14	**-0.27***	-0.15	73	-0.12	-0.12	-0.13
CRF/weight	79	-0.04	0.05	0.21	79	-0.20	-0.19	-0.21
CMR score	76	0.13	**0.32****	**0.25***	75	0.20	0.20	0.15
Inflammatory profile								
C-reactive protein	78	0.01	0.14	0.01	78	0.04	0.04	0.06
IL-2	64	0.01	0.04	0.07	63	-0.03	-0.01	-0.02
IL-4	64	-0.08	-0.05	0.08	63	-0.16	-0.14	-0.11
IL-6	64	-0.09	0.01	0.07	63	-0.02	0.00	-0.02
IL-7	64	-0.19	-0.14	0.04	63	-0.17	-0.15	-0.14
IL-8	64	-0.03	0.10	0.03	63	0.02	0.03	0.01
IL-10	64	0.02	0.10	0.12	63	-0.09	-0.08	-0.13
IL-17a	64	0.07	0.23	0.10	63	-0.09	-0.11	-0.13
IFNɣ	64	0.17	0.24	-0.03	63	0.06	0.07	-0.02
TNFα	64	0.09	0.22	-0.11	63	0.02	0.03	-0.05
Complement 3	78	0.12	0.22	0.01	78	0.09	0.08	0.09
Complement 4	78	0.13	0.12	0.01	78	0.16	0.18	0.18
β-microglobulin 2	78	0.01	0.15	0.02	78	0.02	0.02	0.00
Adiponectin	75	-0.16	-0.16	**-0.24***	74	**-0.25***	**-0.25***	**-0.25***
Leptin	76	-0.01	0.12	0.19	76	**0.26***	0.23	**0.25***

Continuation Table S1. Relationship of neck measures with body composition, the cardiometabolic and inflammatory profile a
confounders (body mass index, body fat percentage, and VAT mass), in women and men.

				MEN			
		Subcutaneous NAT	**Intermuscular NAT**	**Perivertebral NAT**		**Total NAT**	**Total NAT/height**	**NAT p (%)**
Model 1 (adjusted for BMI)								
	n	r	r	r	n	r	r	
Body composition								
BMI (Kg/m²)								
Lean mass (kg)	38	-0.20	-0.21	-0.15	34	-0.25 €	**-0.38***	-0.34 €
Fat mass (kg)	38	0.15	0.31	**0.41***	34	**0.62*****	**0.51****	**0.39***
VAT mass (g	38	0.23	**0.39***	0.12	34	**0.61*****	**0.55*****	**0.40***
Cardiometabolic profile								
Glucose	38	0.10	0.12	0.04	34	**0.48****	**0.44***	**0.36***
Insulin	38	0.12	**0.36***	0.24	34	0.34	0.32	0.19
HOMA	38	0.13	**0.37***	0.23	34	**0.37***	0.34	0.21
Total cholesterol	38	0.08	0.32	0.09	34	0.24	0.25	0.26
LDL-C	38	0.11	**0.35***	0.12	34	0.34	**0.35***	0.31
HDL-C	38	0.09	-0.24	-0.03	34	**-0.39***	**-0.42***	-0.19
TC/HDL-C	38	0.01	**0.45****	0.09	34	**0.50****	**0.53*****	**0.36***
LDL-C/HDL-C	38	0.05	**0.44****	0.11	34	**0.52****	**0.55*****	**0.37***
Triglycerides	38	-0.11	0.29	0.09	34	0.24	0.23	0.23
SBP	37	-0.09	0.01	-0.22	33	0.06	0.07	0.03
DBP	37	-0.12	0.07	-0.12	33	0.16	0.20	0.14
Muscular strength/weight	31	-0.11	**-0.41***	0.01	28	**-0.43***	**-0.42***	**-0.47***
CRF/weight	34	-0.26	**-0.43***	**-0.44***	29	-0.36	-0.33	**-0.47***
CMR score	37	0.03	0.31	0.01	33	**0.54*****	**0.50****	**0.40***
Inflammatory profile								
C-reactive protein	38	0.07	0.04	**0.50****	34	0.08	0.08	-0.01

IL-2	34	0.30	-0.08	-0.10	30	-0.01	0.04	0.24
IL-4	34	**0.50****	0.12	-0.13	30	0.14	0.19	**0.42***
IL-6	34	**0.37***	0.06	0.05	30	0.12	0.17	0.29
IL-7	34	**0.48****	0.12	-0.30	30	0.13	0.18	**0.43***
IL-8	34	**0.57*****	0.25	0.11	30	0.28	0.34	**0.49****
IL-10	34	0.20	0.14	**-0.39***	30	0.20	0.24	0.27
IL-17a	34	0.31	0.10	-0.23	30	0.25	0.29	**0.50****
IFNɣ	34	-0.04	-0.05	-0.12	30	0.12	0.13	0.20
TNFα	34	0.25	0.11	-0.04	30	-0.04	0.01	0.17
Complement 3	38	**0.33***	**0.35***	**0.39***	34	0.27	0.26	0.26
Complement 4	38	-0.17	-0.02	0.29	34	0.03	0.03	-0.02
β-microglobulin 2	38	-0.13	-0.21	0.08	34	0.01	0.01	-0.04
Adiponectin	37	-0.28	**-0.44****	-0.16	33	-0.34	**-0.36***	-0.31
Leptin	37	0.04	0.23	**0.36***	33	0.25	0.23	**0.39***
Model 2 (adjusted for body fat percentage)								
Body composition								
BMI (kg/m$^{2)}$	38	**0.56*****	**0.53*****	0.20	34	**0.58*****	**0.61*****	0.28
Lean mass (kg)	38	0.31	**0.35***	0.15	34	**0.47****	**0.39***	0.12
Fat mass (kg)								
VAT mass (g)	38	**0.48****	**0.52*****	0.06	34	**0.65*****	**0.63*****	0.31
Cardiometabolic profile								
Glucose	38	-0.05	-0.10	-0.15	34	**0.37***	0.33	0.26
Insulin	38	0.23	**0.40***	0.22	34	**0.54*****	**0.52****	0.28
HOMA	38	0.22	**0.39***	0.19	34	**0.56*****	**0.52****	0.29
Total cholesterol	38	0.03	0.22	0.02	34	0.24	0.25	0.25
LDL-C	38	0.04	0.23	0.03	34	0.27	0.28	0.25
HDL-C	38	0.12	-0.12	0.08	34	-0.29	-0.31	-0.09
TC/HDL-C	38	-0.05	0.30	-0.04	34	**0.47****	**0.50****	0.30
LDL-C/HDL-C	38	-0.01	0.29	-0.01	34	**0.44***	**0.46****	0.29
Triglycerides	38	-0.20	0.11	-0.06	34	0.28	0.28	0.21
SBP	37	-0.02	0.08	-0.19	33	0.26	0.26	0.15
DBP	37	0.02	0.18	-0.06	33	**0.51****	**0.53****	**0.35***

Muscular strength/weight	31	-0.13	-0.34	0.17	28	-0.14	-0.15	-0.27
CRF/weight	34	-0.13	-0.24	-0.31	29	0.05	0.07	-0.23
CMR score	37	0.06	0.26	-0.13	33	**0.60*** **	**0.57*** **	**0.37***
Inflammatory profile								
C-reactive protein	38	0.07	0.03	**0.49****	34	0.08	0.08	-0.03
IL-2	34	0.21	-0.11	-0.12	30	-0.05	0.00	0.25
IL-4	34	0.34	0.01	-0.20	30	0.02	0.05	**0.38***
IL-6	34	0.29	0.04	0.04	30	0.07	0.11	0.30
IL-7	34	**0.35***	0.04	**-0.36***	30	-0.01	0.03	**0.38***
IL-8	34	**0.40***	0.12	0.05	30	0.22	0.26	**0.50****
IL-10	34	0.01	-0.06	**-0.51****	30	0.08	0.11	0.21
IL-17a	34	0.12	-0.09	**-0.38***	30	-0.01	0.04	**0.37***
IFNɣ	34	-0.30	**-0.37***	**-0.35***	30	-0.30	-0.29	-0.07
TNFα	34	0.13	0.02	-0.08	30	0.02	0.07	0.22
Complement 3	38	**0.37***	**0.36***	**0.36***	34	0.31	0.31	0.24
Complement 4	38	-0.09	0.00	0.27	34	0.01	0.02	-0.07
β-microglobulin 2	38	-0.18	-0.24	0.08	34	0.10	0.09	0.02
Adiponectin	37	-0.27	**-0.39***	-0.12	33	-0.27	-0.29	-0.23
Leptin	37	0.14	0.22	0.28	33	0.11	0.12	0.25
Model 3 (adjusted for VAT mass)								
Body composition								
BMI (kg/m$^{2)}$	38	**0.37***	**0.32***	0.25	34	0.15	0.23	0.05
Lean mass (kg)	38	0.05	0.04	0.01	34	-0.13	-0.21	-0.26
Fat mass (kg)	38	0.19	0.24	**0.48****	34	**0.37***	0.26	0.18
VAT mass (g)								
Cardiometabolic profile								
Glucose	38	0.03	0.02	0.00	34	0.34	0.28	0.25
Insulin	38	0.15	**0.37***	0.26	34	0.27	0.26	0.11
HOMA	38	0.16	**0.36***	0.25	34	0.29	0.26	0.12
Total cholesterol	38	-0.08	0.14	0.00	34	0.07	0.08	0.16
LDL-C	38	-0.05	0.16	0.03	34	0.16	0.17	0.20

HDL-C	38	0.10	-0.20	-0.02	34	**-0.44***	**-0.45****	-0.17
TC/HDL-C	38	-0.14	0.28	0.00	34	**0.40***	**0.44***	0.27
LDL-C/HDL-C	38	-0.10	0.26	0.03	34	**0.40***	**0.43***	0.27
Triglycerides	38	-0.21	0.17	0.03	34	0.18	0.19	0.18
SBP	37	-0.14	-0.06	-0.25	33	-0.08	-0.06	-0.05
DBP	37	-0.29	-0.15	-0.22	33	0.01	0.07	0.04
Muscular strength/weight	31	-0.09	**-0.36***	0.01	28	-0.26	-0.25	-0.37
CRF/weight	34	-0.10	-0.24	**-0.37***	29	-0.14	-0.11	-0.36
CMR score	37	-0.08	0.17	-0.04	33	**0.39***	**0.36***	0.27
Inflammatory profile								
C-reactive protein	38	0.08	0.04	**0.50****	34	0.04	0.03	-0.05
IL-2	34	0.27	-0.10	-0.11	30	0.08	0.13	0.31
IL-4	34	**0.35***	-0.03	-0.21	30	0.12	0.16	**0.42***
IL-6	34	0.30	0.01	0.01	30	0.21	0.25	0.35
IL-7	34	0.34	-0.02	**-0.37***	30	0.06	0.10	**0.41***
IL-8	34	**0.45****	0.14	0.04	30	0.32	**0.37***	**0.52****
IL-10	34	0.07	0.00	**-0.45****	30	0.11	0.15	0.22
IL-17a	34	0.21	-0.01	-0.28	30	0.19	0.23	**0.48****
IFNɣ	34	-0.08	-0.10	-0.15	30	0.08	0.08	0.18
TNFα	34	0.25	0.14	-0.04	30	0.05	0.11	0.23
Complement 3	38	**0.35***	**0.36***	**0.40***	34	0.21	0.22	0.21
Complement 4	38	-0.10	0.03	0.32	34	0.04	0.06	-0.02
β-microglobulin 2	38	0.00	-0.04	0.16	34	0.21	0.20	0.07
Adiponectin	37	-0.30	**-0.46****	-0.18	33	**-0.47****	**-0.48****	-0.35
Leptin	37	0.18	0.33	**0.42***	33	0.34	0.33	**0.40***

Pearson correlation coefficient is provided for women and men. All the variables related to NAT and CMR ((except for muscula
fitness) were transformed (square root transformation) in order to make its distribution closer to the normal distribution. Statist
indicated in bold: *P≤0.05, **P≤0.01, ***P≤0.001. BMI: body mass idex, CRF: cardiorespiratory fitness, CRF: cardiorespiratory fitn
DBP: diastolic blood pressure, HDL: high-density lipoprotein-cholesterol, HOMA: homeostatic model assessment of insulin res
interferon gamma, LDL-C: low-density lipoprotein-cholesterol, LDL-C/HDL-C: low-density lipoprotein cholesterol (LDL-C)/HD

activity, MPA: moderate physical activity, MVPA: moderate-vigorous physical activity, MVPA10min: moderate-vigorous physi
minutes, NAT: neck adipose tissue, PA: physical activity TC/HDL-C: total cholesterol (TC)/high-density lipoprotein cholesterol
necrosis factor-alpha, SBP: systolic blood pressure, VPA: vigorous physical activity, VAT: visceral adipose tissue. Significant dif
asterisks: *P≤0.05, **P≤0.01, ***P≤0.001.

Table S2. Relationship of anthropometric and body composition parameters with the cardiometabolic profile, in women and m

	BMI (Kg/m²)				**WC (cm)**				**Neck**
	Women		Men		Women		Men		Women
	r	n	r	n	r	n	r	n	r
Glucose	**0.24***	93	**0.52*****	44	**0.31****	93	**0.60*****	44	**0.32***
Insulin	**0.46*****	93	**0.68*****	44	**0.50*****	93	**0.64*****	44	**0.54*****
HOMA	**0.46*****	93	**0.67*****	44	**0.50*****	93	**0.65*****	44	**0.55*****
Total cholesterol	-0.098	93	**0.39***	44	-0.05	93	**0.34***	44	0.12
LDL-C	0.054	93	**0.39***	44	0.08	93	**0.35***	44	0.21
HDL-C	**-0.41*****	93	**-0.30***	44	**-0.33*****	93	**-0.33***	44	-0.20
TC/HDL-C	**0.34*****	93	**0.49****	44	**0.30****	93	**0.47*****	44	**0.32***
LDL-C/HDL-C	**0.32***	93	**0.46*****	44	**0.28****	93	**0.45****	44	**0.32***
Triglycerides	0.17	93	**0.47*****	44	**0.22***	93	**0.44****	44	0.23
SBP	**0.40*****	94	0.35*	43	**0.39*****	94	0.28	43	**0.47*****
DBP	0.18	94	**0.37***	43	0.20	94	**0.31***	43	0.14
Muscular strength/weight	**-0.73*****	85	**-0.65*****	37	**-0.56*****	85	**-0.73*****	37	**-0.39****
Muscular $strength_{LM}$	**-0.48*****	85	-0.28	37	**-0.36*****	85	**-0.39***	37	**-0.26***
CRF/weight	**-0.48*****	94	**-0.58*****	39	**-0.43*****	94	**-0.59*****	39	**-0.41*****
CRF_{LM}	-0.03	94	-0.27	39	-0.11	94	-0.29	39	-0.22
CMR score	**0.64*****	90	**0.71*****	43	**0.73*****	90	**0.75*****	43	**0.61*****

Continuation of tabla S2. Relationship of anthropometric and body composition parameters with the cardiometabolic profile, i

	Fat mass total (Kg)				Fat mass percentage (%)				
	Women		Men		Women		Men		Wom
	r	n	r	n	r	n	r	n	r
Glucose	**0.21***	93	**0.62*****	44	0.14	93	**0.60*****	44	**0.25***
Insulin	**0.44*****	93	**0.69*****	44	**0.38*****	93	**0.59*****	44	**0.27****
HOMA	**0.43*****	93	**0.69*****	44	**0.36*****	93	**0.60*****	44	**0.29****
Total cholesterol	-0.08	93	**0.44****	44	-0.05	93	**0.42****	44	-0.08
LDL-C	0.06	93	**0.45****	44	0.05	93	**0.45****	44	0.038
HDL-C	**-0.39*****	93	**-0.33***	44	**-0.35*****	93	**-0.37***	44	**-0.21***
TC/HDL-C	**0.33*****	93	**0.54*****	44	**0.31****	93	**0.55*****	44	0.16
LDL-C/HDL-C	**0.31****	93	**0.52*****	44	**0.27***	93	**0.54*****	44	0.19
Triglycerides	0.18	93	**0.52*****	44	**0.21***	93	**0.51*****	44	-0.002
SBP	**0.39*****	94	**0.36***	43	**0.27****	94	**0.30***	43	**0.42*****
DBP	0.19	94	**0.38***	43	0.13	94	**0.31***	43	0.16
Muscular strength/weight	**-0.77*****	85	**-0.72*****	37	**-0.76*****	85	**-0.74*****	37	**-0.36*****
Muscular strength$_{LM}$	**-0.47*****	85	-0.30	37	**-0.36*****	85	-0.29	37	**-0.40*****
CRF/weight	**-0.52*****	94	**-0.66*****	39	**-0.52*****	94	**-0.67*****	39	**-0.23***
CRF$_{LM}$	-0.01	94	-0.27	39	0.07	94	-0.26	39	-0.141
CMR score	**0.61*****	90	**0.77*****	43	**0.50*****	90	**0.73*****	43	**0.47*****

Continuation of tabla S2. Relationship of anthropometric and body composition parameters with the cardiometabolic profile, i

	VAT mass (g)				Total NAT				
	Women		Men		Women		Men		Wom
	r	n	r	n	r	n	r	n	r
Glucose	**0.26***	93	**0.60*****	44	**0.25***	78	**0.63*****	34	0.22
Insulin	**0.49*****	93	**0.72*****	44	**0.59*****	78	0.68***	34	**0.41*****
HOMA	**0.49*****	93	**0.73*****	44	**0.57*****	78	**0.69*****	34	**0.41*****
Total cholesterol	-0.02	93	**0.54*****	44	-0.13	78	0.46**	34	0.02
LDL-C	0.06	93	**0.53*****	44	-0.04	78	0.48**	34	0.13
HDL-C	**-0.33*****	93	**-0.32***	44	**-0.39*****	78	**-0.53*****	34	**-0.37*****
TC/HDL-C	**0.33*****	93	**0.63*****	44	**0.27***	78	**0.73*****	34	**0.39*****
LDL-C/HDL-C	**0.28****	93	**0.60*****	44	0.21	78	**0.68*****	34	**0.34****
Triglycerides	**0.33*****	93	**0.60*****	44	**0.27***	78	**0.63*****	34	**0.37*****
SBP	**0.42*****	94	**0.39****	43	**0.33****	79	0.28	33	**0.38*****
DBP	**0.27****	94	**0.51*****	43	**0.29****	79	**0.38***	33	**0.22***
Muscular strength/weight	**-0.67*****	85	**-0.65*****	37	**-0.59*****	73	**-0.65*****	28	**-0.62*****
Muscular strength$_{LM}$	**-0.41*****	85	-0.26	37	**-0.36****	73	-0.31	28	**-0.41*****
CRF/weight	**-0.55*****	94	**-0.62*****	39	**-0.57*****	79	**-0.43***	29	**-0.36*****
CRF$_{LM}$	-0.13	94	-0.28	39	-0.18	79	0.03	29	-0.002
CMR score	**0.68*****	90	**0.81*****	43	**0.62*****	75	**0.82*****	33	**0.66*****

Pearson correlation coefficients were determined to examine of anthropometric and body composition parameters with the card
and men.*P≤0.05, **P≤0.01, ***P≤0.001. All NAT and cardiometabolic profile variables (except for muscular and cardiorespirator
transformed to bring their distributions closer to normal. CRF: cardiorespiratory fitness, CRF: cardiorespiratory fitness relative
diastolic blood pressure, HDL: high-density lipoprotein-cholesterol, HOMA: homeostatic model assessment of insulin resistanc
lipoprotein-cholesterol, LDL-C/HDL-C: low-density lipoprotein cholesterol (LDL-C)/HDL-C ratio, TC/HDL-C: total cholesterol
cholesterol (HDL-C) ratio, SBP: systolic blood pressure, VAT: visceral adipose tissue.

Table S3. Relationship of anthropometric and body composition parameters with the inflammatory profile, in women and men

	BMI (Kg/m²)				**WC (cm)**				**Neck c**	
	Women		Men		Women		Men		Women	
	r	n	r	n	r	N	r	n	r	N
C-reactive protein	**0.26***	93	0.27	44	**0.28****	93	0.21	44	0.12	5
IL-2	-0.07	76	0.01	38	-0.14	76	0.11	38	0.08	4
IL-4	-0.04	76	0.01	38	-0.06	76	0.10	38	0.13	4
IL-6	0.01	76	0.10	38	-0.04	76	0.14	38	0.17	4
IL-7	**-0.23***	76	0.05	38	-0.22	76	0.15	38	-0.15	4
IL-8	0.05	76	0.14	38	0.01	76	0.21	38	0.02	4
IL-10	0.06	76	0.06	38	-0.01	76	0.11	38	0.04	4
IL-17a	-0.04	76	-0.06	38	-0.09	76	0.12	38	-0.01	4
IFNɣ	0.09	76	-0.11	38	0.01	76	0.05	38	-0.03	4
TNFα	0.15	76	0.14	38	0.09	76	0.16	38	0.19	4
Complement 3	**0.40*****	93	**0.68*****	44	**0.48*****	93	**0.65*****	44	**0.40****	5
Complement 4	**0.32****	93	**0.33***	44	**0.32****	93	0.24	44	0.21	5
β-microglobulin 2	0.05	93	-0.17	44	0.14	93	-0.27	44	**-0.27***	5
Adiponectin	-0.19	89	**-0.46****	43	-0.14	89	**-0.49*****	43	-0.15	5
Leptin	**0.62*****	91	**0.77*****	43	**0.49*****	91	**0.76*****	43	**0.37****	5

Continuation of table S3. Relationship of anthropometric and body composition parameters with the inflammatory profile, in w

	Fat mass (kg)				**Fat mass percentage (%)**				
	Women		Men		Women		Men		Wom
	r	n	r	n	r	n	r	n	r
C-reactive protein	**0.32****	93	**0.31***	44	**0.30****	93	0.27	44	0.19
IL-2	-0.14	76	-0.06	38	-0.07	76	0.01	38	-0.16
IL-4	-0.07	76	-0.05	38	-0.02	76	0.03	38	-0.12
IL-6	-0.03	76	0.01	38	0.03	76	0.05	38	-0.11
IL-7	**-0.26***	76	0.00	38	-0.18	76	0.08	38	**-0.26***
IL-8	0.04	76	0.07	38	0.17	76	0.13	38	-0.15
IL-10	0.02	76	0.08	38	0.05	76	0.12	38	-0.01
IL-17a	-0.04	76	0.00	38	-0.01	76	0.11	38	-0.04
IFNɣ	0.03	76	0.05	38	0.00	76	0.15	38	0.10
TNFα	0.11	76	0.09	38	0.13	76	0.11	38	0.06
Complement 3	**0.44*****	93	**0.71*****	44	**0.44*****	93	**0.67*****	44	**0.23***
Complement 4	**0.31****	93	**0.32***	44	**0.37*****	93	**0.36***	44	0.06
β-microglobulin 2	0.09	93	-0.20	44	0.05	93	-0.21	44	0.15
Adiponectin	-0.17	89	-0.44	43	-0.20	89	**-0.47*****	43	-0.04
Leptin	**0.69*****	91	**0.79*****	43	**0.66*****	91	**0.82*****	43	**0.36*****

Continuation of table S3. Relationship of anthropometric and body composition parameters with the inflammatory profile, in

	VAT mass (g)				**Total NAT**				
	Women		Men		Women		Men		Wom
	r	n	r	n	r	n	r	n	r
C-reactive protein	**0.32****	93	0.25	44	**0.39*****	78	0.19	34	**0.38*****
IL-2	-0.09	76	0.02	38	-0.06	63	-0.09	30	-0.17
IL-4	-0.01	76	0.06	38	-0.06	63	-0.05	30	-0.12
IL-6	0.02	76	0.07	38	0.05	63	-0.05	30	-0.06
IL-7	-0.20	76	0.11	38	-0.23	63	-0.04	30	**-0.29***
IL-8	0.05	76	0.17	38	0.07	63	0.17	30	0.02
IL-10	-0.02	76	0.14	38	-0.03	63	0.1	30	0.02
IL-17a	-0.09	76	0.09	38	-0.09	63	0.12	30	0.06
IFNɣ	-0.06	76	0.00	38	0.02	63	-0.01	30	0.11
TNFα	0.03	76	0.15	38	0.08	63	0.05	30	0.14
Complement 3	**0.50*****	93	**0.67*****	44	**0.46*****	78	**0.62*****	34	**0.48*****
Complement 4	**0.39*****	93	**0.30***	44	**0.39*****	78	0.31	34	**0.32****
β-microglobulin 2	-0.02	93	-0.24	44	0.19	78	0.01	34	**0.28***
Adiponectin	**-0.24***	89	**-0.43****	43	**-0.31****	74	**-0.56*****	33	**-0.31****
Leptin	**0.59*****	91	**0.69*****	43	**0.59*****	76	**0.69*****	33	**0.46*****

Pearson correlation coefficients were determined to examine the association between anthropometric and body composition par
profile, in women and men. *P≤0.05, **P≤0.01, ***P≤0.001. All NAT and cardiometabolic profile variables were square r
distributions closer to normal. IL: interleukin, IFNɣ: interferon gamma, TNFα: tumour necrosis factor-alpha, VAT: visceral adip

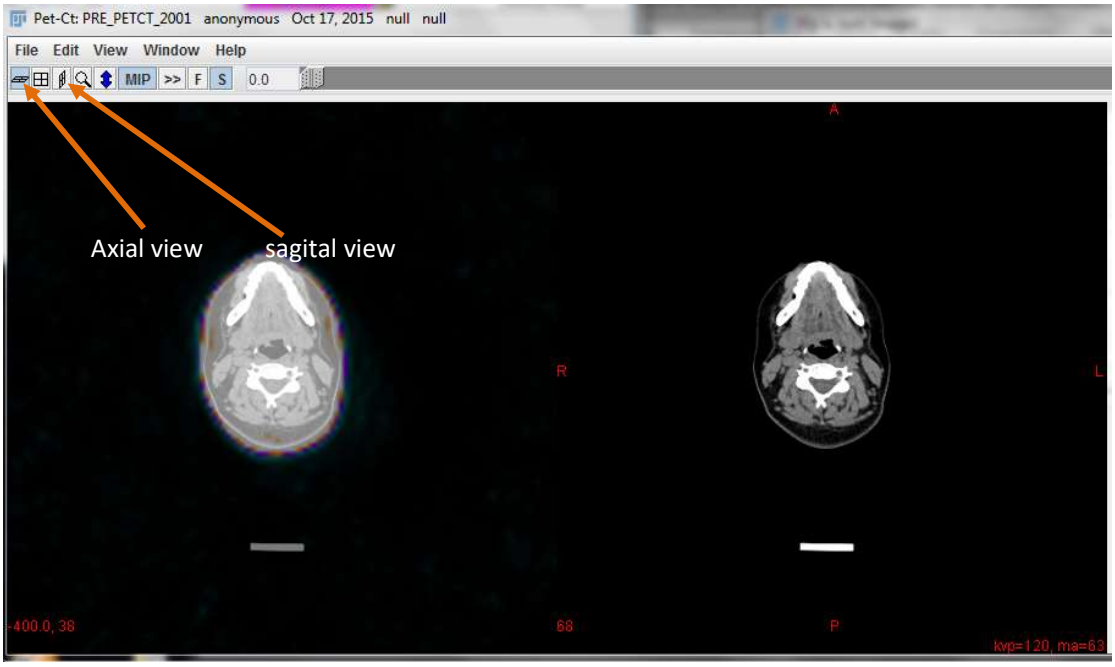
PROTOCOL USED TO ANALYSE AND QUANTIFY NECK BODY COMPOSITION

For the current study aims we performed different analyses in order to measure neck adipose tissue (NAT) accumulation and its distribution among tissue compartments. We additionally analysed neck body composition (and within it, the total NAT). We provide below the protocol that we followed for these purposes.

A) NECK ADIPOSE TISSUE COMPARTMENTS (ROIs 1, 2a, 2b and 3)

We firstly need to open the FIJI software http://sourceforge.net/projects/bifijiplugins/ that was used to analyse the PET/CT images. Once opened, we can load the images that we want to analyse. In the case you do not have this program, it can be downloaded for free in the above link.

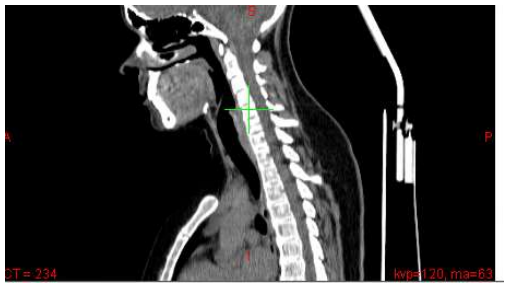
Then, we need to define the NAT compartments. To do that we draw different ROIs at an axial view:



2. To make comparisons possible with previous studies, and because normally the neck circumference measure is performed at the laryngeal prominence, which is approximately at the level of C5, we measured NAT at this level. Therefore, we are going to stablish all these ROIs at the vertebra C5 level. To do that:

    a. We need to count the number of cervical vertebras in the sagittal view as the in the image below.

    b. When we found the vertebra C5, we need to click with the mouse on this vertebra (see an example in the image below):



Sagital view

    c. Then, we need to move into the **axial** plane. From the axial view, we will draw the different ROIS for quantifying the adipose tissue of the neck area.

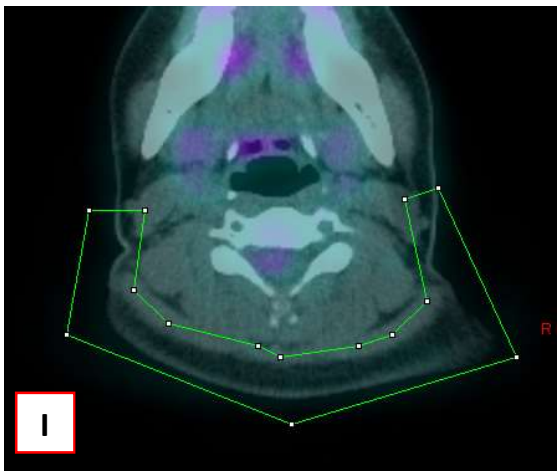
IMPORTANT: For every ROI, we will define an upper and lower boundary of one slice each one. For example: If we are at the C5 level, which is placed at the height of 35th slice, we must choice in the "slice limits" one slice more for the upper and lower parts (i.e. 34 and 36).

ROIs delimitation.

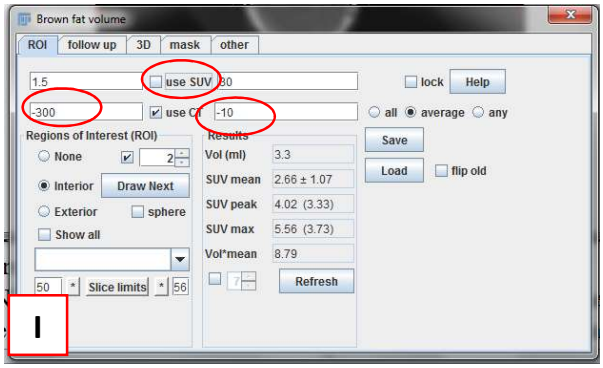
Then, we will delineate the ROIS for each NAT compartment, following the procedure developed by Torriani et al. (see method section in the main manuscript):

I: *Subcutaneous* NAT. This ROI will be drawn in the posterior neck, between the skin and deep cervical fascia.

1. Axial display
2. Edit → Brown fat, ROIs
3. Draw ROI
4. Include upper and down slices.
5. Write the name of the ROI (1) in the white box.
6. Once that the ROI is perfectly drawn, we will need to apply different thresholds in order to quantify the specific tissues that there are inside this ROI.

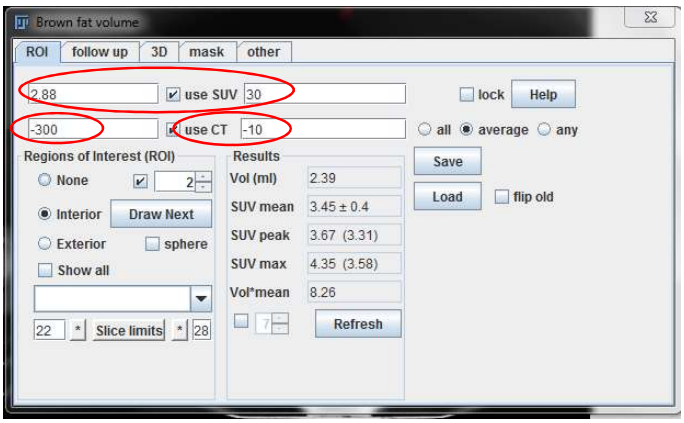


a. For the quantification of subcutaneous NAT, will select a radiodensity of -300 -10 HU, **without** selecting any SUV. Then, we will obtain the volume (ml), SUV mean and SUV peak of the subcutaneous NAT.



IMPORTANT: Every time that we apply a new criteria, we need to clik in "Refresh" for update the settings.

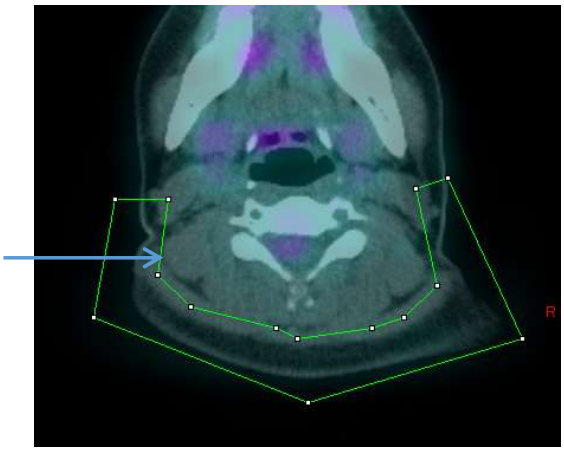
b. As a sensitivity analysis, we also will quantify brown adipose tissue (BAT) within this ROI. For this purpose, we will select as previously, a radiodensity of -300 -10 HU, but now it will be selected the individualized **SUV** [calculated as 1.2/ (lean body mass/body mass)]. Then, we will obtain the volume (ml), SUVmean and SUVpeak.

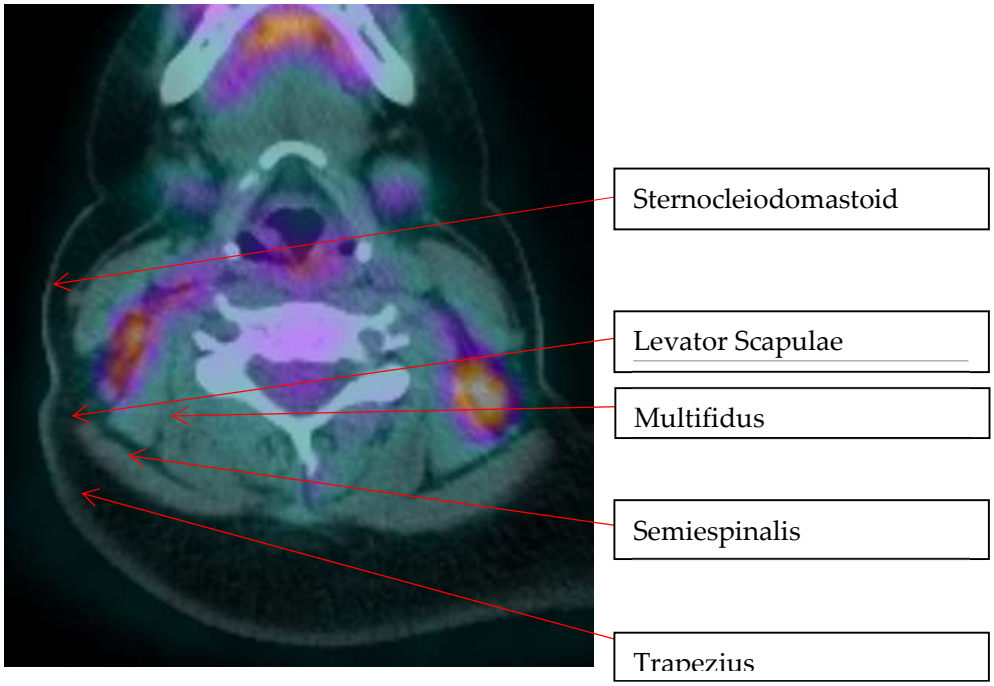


Summary table			
ROI 1	**HU**	**SUV**	**SLICES**
SUBCUTANEOUS NAT	-300, -10	NA	1 up 1 down
SUBCUTANEOUS BAT	-300, -10	IND	1 up 1 down

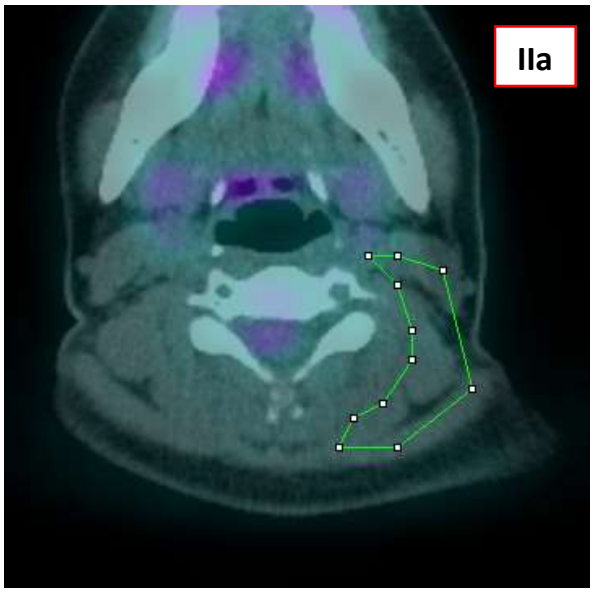
II: *Intermuscular* NAT: This compartment must include the space placed between the *Sternocleidomastoid*, *Levator Scapulae*, *Semiespinalis* and *Trapezius* muscles, separated from the subcutaneous fat by the deep cervical fascia. In this case, we will draw 2 different ROIs, one for the left side, and another one for the right side. Then, we will obtain the average intermuscular NAT as the mean of both ROIs.

It is important to be sure that **there is no overlapping between the intermuscular and subcutaneous NAT ROI**. To minimize this, the vertical line between *trapezius* and *sternocleidomastoid* has to be in the exterior edge of *Levator Scapulae*:





1. Axial display
2. Draw next (left ROI)
3. Include upper and down slices.
4. Write the name of the left ROI (2a) in the white box.
5. Once that the left ROI is perfectly drawn, we need to apply different thresholds in order to quantify the specific tissues that there are within this ROI.
6. We will do the same procedure with the ROI from the right side:
7. Draw next (right ROI)
8. Include upper and down slices.
9. Write the name of the right ROI (2b) in the white box.
10. Once that the ROI is perfectly drawn, we need to apply different thresholds in order to quantify the specific tissues that there are within this ROI.



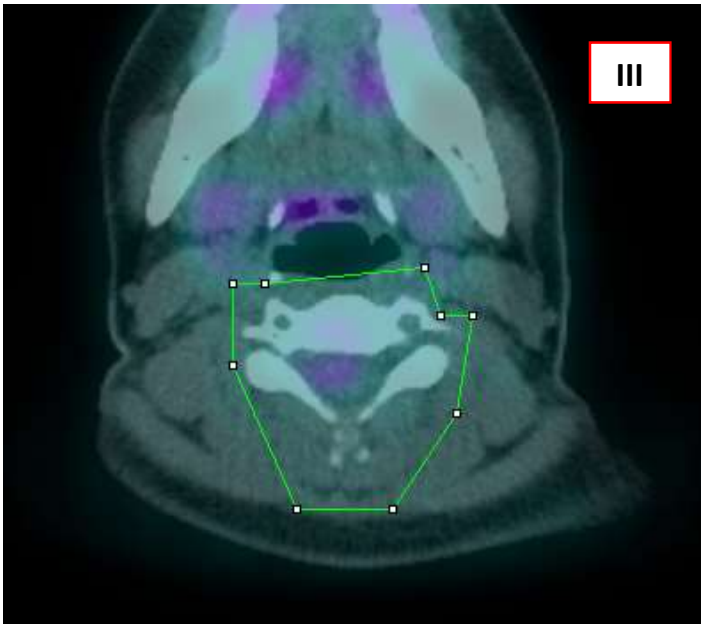
a. For the quantification of the intermuscular NAT in both ROIs, we will select a radiodensity of -300 -10 HU, **without** selecting any SUV. Then, we will obtain the volume (ml), SUV mean and SUV peak of these ROIs. Finally, we will calculate an average of the parameters obtained in the left and right ROIs of the intermuscular NAT.

b. As a sensitivity analysis, we also will quantify brown adipose tissue (BAT) within these ROIs. For this purpose, we will select as previously, a radiodensity of -300 -10 HU, but now it will be selected the individualized **SUV** [calculated as 1.2/ (lean body mass/body mass)]. Then, we will obtain the volume (ml), SUV mean and SUV peak. Then, we will obtain an average of both ROIs.

Summary table			
ROI 2a	**HU**	**SUV**	**SLICES**
LEFT INTERMUSCULAR NAT	-300, -10	NA	1 up 1 down
LEFT INTERMUSCULAR BAT	-300, -10	IND	1 up 1 down
ROI 2b	**HU**	**SUV**	
RIGHT INTERMUSCULAR NAT	-300, -10	NA	1 up 1 down
RIGHT INTERMUSCULAR BAT	-300, -10	IND	1 up 1 down

III: *Perivertebral NAT*: This ROI will be drawn around the cervical vertebrae number 5. We should include all the fat tissue interspersed between cervical muscles surrounding the cervical vertebrae, and between trapezius and multifidus.

1. Axial display
2. Draw next
3. Include upper and down slices.
4. Write the name of the ROI (3) in the white box.
5. Once that the ROI is perfectly draw, we need to apply different thresholds in order to quantify the different tissue that there is inside every ROI.



a. For the quantification of the perivertebral NAT, will select a radiodensity of -300 -10 HU, **without** selecting any SUV. Then, we will obtain the volume (ml), SUVmean and SUVpeak of the perivertebral NAT.

b. As a sensitivity analysis, we also will quantify brown adipose tissue (BAT) within this ROI. For this purpose, we will select as previously, a radiodensity of -300 -10 HU, but now it will be selected the individualized **SUV** [calculated as 1.2/ (lean body mass/body mass)]. Then, we will obtain the volume (ml), SUVmean and SUVpeak.

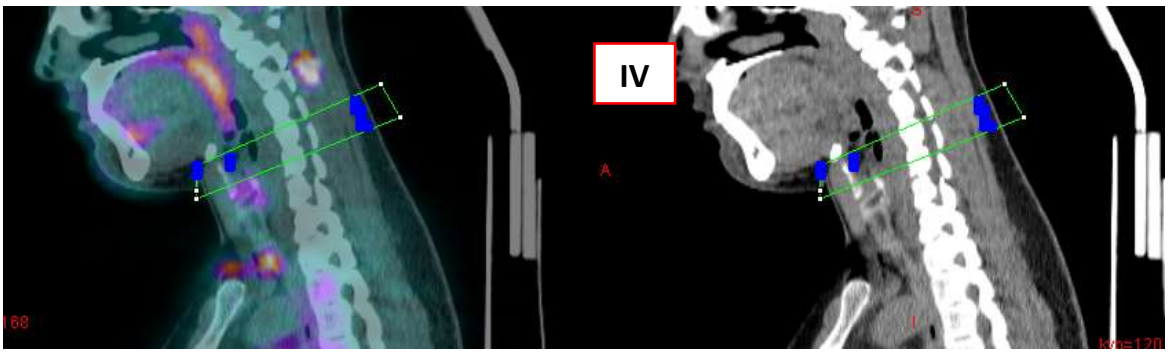
Summary table			
ROI 3	**HU**	**SUV**	**SLICES**
PERIVERTEBRAL NAT	-300, -10	NA	1 up 1 down
PERIVERTEBRAL BAT	-300, -10	IND	1 up 1 down

B) NECK BODY COMPOSITION (ROI 4)

Now we are going to draw a single slice-ROI at the level of C5 in order to determine neck body composition, and the tissues contained within this ROI. We will determine the "total" NAT comprised in this ROI (without differentiating between compartments). For this purpose:

1. Set sagital view.
2. We have to place the ROI at the C5 level. Click on the 5th vertebra body.
3. In order to take a central segment, we will select the "coronal display". Click over the middle of the body of the 5th vertebra. Then, select the sagital display again.

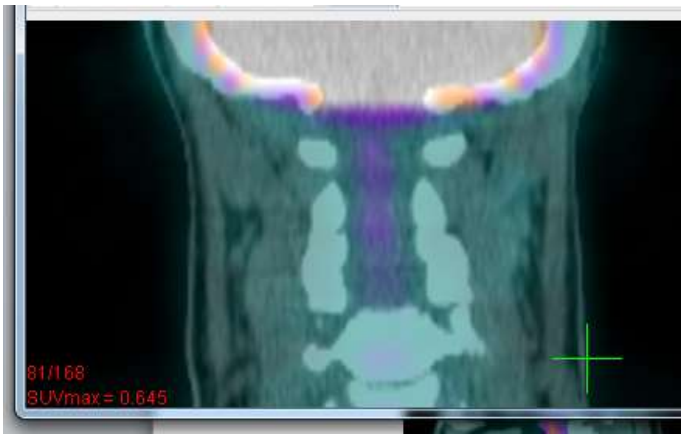
4. Draw an oblique ROI (parallel to the body axis), taking the superficial skin edge. This ROI should include the whole neck circumference (i.e., all its length and width), and its upper and lower boundaries would extend from the upper part of the C5 vertebral body to its lowest part, respectively. **NOTE**: due to the position of some subjects it is possible that you take some "jowl". In that case, please note in the provided excel sheet that it was not possible to draw this ROI without including the jowl (this will be required for posterior sensitivity analyses).



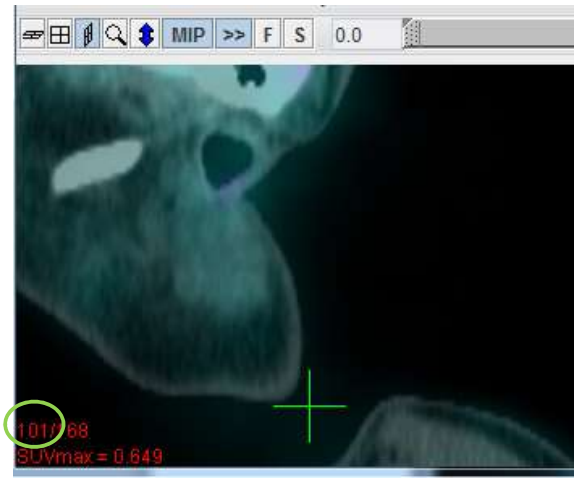
5. As previously stated you would have to include all the neck width (go to the coronary display and select the two ends of the neck), **except in the next case**: some participants have their shoulders elevated, so we must be careful in order not to include them within the ROI.

a. Set "coronal view"

b. Click in the limit of the neck with the shoulder:



c. Set sagittal view and annotate the number of slice



d. Repeat with the other side

e. Now we know the number of slices to take above and below the vertebrae C5.

6. Finally, once that the ROI is perfectly drawn, we will need to apply different thresholds in order to quantify the specific tissues that there are within this ROI(1; 2).

   a. For the quantification of the **total NAT**, we will select a radiodensity of -300 -10 HU, **without** selecting any SUV. Then, we will obtain the volume (ml), SUV mean and SUVpeak.

   b. As a sensitivity analysis, we also will quantify BAT within this ROI. For this purpose, we will select as previously, a radiodensity of -300 -10 HU, but now it will be selected the individualized **SUV** [calculated as 1.2/(lean body mass/body mass)]. Then, we will obtain the volume (ml), SUVmean and SUVpeak.

c. For the quantification of the neck lean tissue, we will select a radiodensity of -9/150 HU, **without** selecting any individualized SUV. Then, we will obtain the volume (ml), SUVmean and SUVpeak.

Summary table			
ROI 4	**HU**	**SUV**	**SLICES**
Total NAT at C5	-300, -10	NA	Upper and lower boundaries of vertebrae C5
BAT at C5	-300, -10	IND	Upper and lower boundaries of vertebrae C5
Lean tissue at C5	-9, 150	NA	Upper and lower boundaries of vertebrae C5

d. For the quantification of the bone tissue, we will select a radiodensity of 151/1000 HU, **without** selecting any SUV. Then we will obtain the volume (ml), SUVmean and SUVpeak

Within this ROI, we will also aim to quantify the percentage of total NAT, i.e., the mass of tissue within this ROI (comprising neck adipose, lean and bone tissue) that can be classified as NAT. For this purpose, we will apply the following protocol:

a. We will use specific reference densities (obtained from the International Commission on Radiological Protection, ICRP) for each tissue based on its location (i.e., neck fat: 0.95g/cm^3, neck muscle: 1.05 g/cm^3, bone mass: 1.135 g/cm^3) (42;43).

b. As the volumetric CT assessment allows to estimate the total NAT volume for a specific ROI, and we have the reference densities of our interest to apply, we will estimate the amount of mass corresponding to each tissue within this ROI.

c. Once we have calculated this, we will proceed to calculate the relative amount of NAT compared to the whole tissue mass in this ROI, obtaining the percentage of total NAT.

Intra-assay coefficients of variation for cytokines

Using the results from a subsample of the present subjects, intra-assay coefficients of variation (CVs) were calculated for the set of cytokines studied: IL-2, 13%; IL-4, 8.7%; IL-6, 7.7%; IL-7, 7.3%; IL-8, 6.6%; IL-10, 20.7%; IL-17a, 8.6%; IFNɣ, 10.3%; and TNF-α, 8.3%. The CVs for leptin and adiponectin were 9% and 7.8% respectively.

Subjects excluded due to problems with image analysis

i) Twenty one subjects (15 women and 6 men) were excluded from the analysis of the subcutaneous and intermuscular neck adipose tissue (NAT), and 22 subjects (16 women and 6 men) from the analysis of the perivertebral NAT, due to image analyses problems. The most common problem was in subjects with excess upper body fat, in whom ROIs for distinguishing specific compartments could not be accurately drawn. Their CT scan resolutions were also poor.

ii) Twenty five subjects (15 women and 10 men) were excluded from 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.

Sensitivity analyses

Analysis taking into account all subjects originally excluded due to NAT quantification problems

Tests were performed to determine whether the relationships between the compartmental and total NAT volumes with body composition, CMR and inflammatory markers varied after taking into account the subjects originally excluded. Overall, no changes were found in the association between the NAT volumes and body composition, although changes in the strength of the relationship between the NAT volumes and the cardiometabolic and

inflammatory markers were observed, both in women and men (data not shown). Further analyses therefore involved only those subjects in whom the compartmental and total NAT volumes could be accurately estimated.

Associations between neck measurements and body composition, cardiometabolic risk, and inflammatory variables after adjusting for brown adipose tissue volume

The neck region is comprised of a great diversity of tissues (11), including brown adipose tissue (BAT). Beyond the classical white adipose tissue, the ectopic accumulation of which has been related to CMR, BAT has been hypothesized as a potential target for combating obesity and diabetes given its ability to oxidize glucose and lipids (44). Since the radiodensity range used to determine NAT also included BAT (45), and since the white and brown fat within this area may have opposing roles in cardiometabolic health, the BAT volume and its metabolic activity were determined for use in sensitivity analyses, i.e., to determine whether the relationship between the NAT measurements and body composition, CMR and inflammatory variables remained similar after adjustment for BAT volume. Briefly, those pixels in the above mentioned ROIs that fell within the fixed radiodensity range (-300 to -10 HU), and had a standardized uptake value (SUV) above the individualized 18F-FDG SUV threshold [calculated as 1.2/ (lean body mass/body mass)] (46), were deemed to represent BAT. This made no difference to the results for the women, but slightly changed the relationship between the NAT measurements and CMR and inflammatory variables in the men, probably due to a reduction in the sample size (data not shown).

Analysis taking into account a potential outlier for the compartmental NAT volume results

One male participant had compartmental NAT volumes that exceeded those of the other subjects (subcutaneous NAT: 16.2 mL, z score = 3.58; intermuscular NAT: 3.1 mL, z score=2.61; and perivertebral NAT: 1.8 mL, z score= 3.92). These values were not caused by any methodological mistake; the subject was simply obese and had the largest NC. Since these values are biologically plausible, and the participant had a total NAT volume within the normal range, all the analyses were re-run including this participant. Overall the results were replicated, although the relationship between the subcutaneous NAT volume and lean mass became non-significant. The same was seen with respect to a few CMR factors (data not shown).

Data consistency and inter-evaluator estimate differences

Coefficient of Variation (CV)

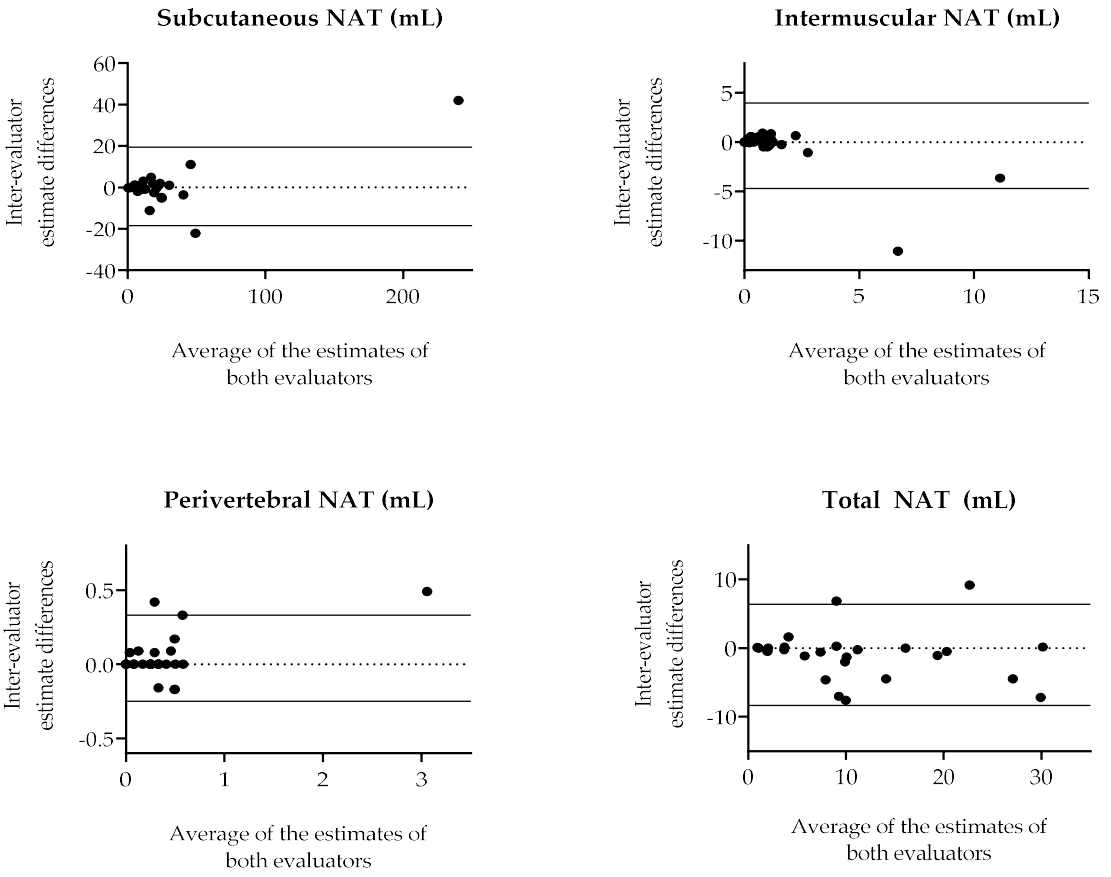
To examine how consistent (i.e., the uniformity in the values of the data/distribution with respect the mean) was the data related to NAT, we estimated the CV for the compartmental and total NAT volumes. The CV for NAT measures was as follow: subcutaneous (130.7%), intermuscular (107.6%), perivertebral (99.5%), and total (90.9%). Of note, as explained above, there was an outlier participant who presented larger compartmental NAT volumes with respect their counterparts. When this participant was excluded from the analyses, the CV for the compartmental NAT measures was as follow: subcutaneous (84.5%), intermuscular (100.7%), and perivertebral (77.1%).

As can be observed the consistency of our data seems to be low, which might be explained by a high biological variability in NAT accumulation and distribution, or by methodological issues. Interestingly, the NAT measures with the lowest CV were the perivertebral and the total NAT volumes, which seems coherent, since

the former variable was delineated with the smallest ROI, and the latter with a fixed ROI (i.e., both are likely to diminish the variability of the measure). Nonetheless, the subcutaneous and intermuscular NAT volumes, whose ROIs are more difficult to draw due to the CT scan resolution, the difficulties to identify certain anatomical spots, and the potential overlapping of both compartments, presented the highest CVs. This information is valuable and should be consider when interpreting the current results. Future methodological studies should aim to find accurate protocols/methods to analyse NAT.

Bland-Altman plots

To understand the comparability of the results when NAT volumes are estimated by different evaluators, we performed Bland-Altman plots of these variables in a subgroup of participants from the present cohort. Two researchers independently analysed the PET/CT images of several participants to estimate compartmental NAT (n=30) and total NAT (n=26). Then, the average of the estimates obtained by both evaluators was plotted against the inter-evaluator estimate differences in NAT volumes assessment, and the limits of agreements were calculated.



As can be observed, in general terms there was a good agreement between NAT estimates performed by different evaluators, although some bias could be observed, which was mainly explained for the above mentioned outlier. In addition, few points surpassed the limits of agreement. Whether discrepancies in NAT estimates between evaluators are clinically relevant is difficult to know given the still immature body of evidence in this field. No significant differences ($P<0.05$) were observed when the mean of NAT measures obtained by both evaluators was compared. When the outlier participant was excluded from the analysis, no relationship was found ($P<0.05$) between the average of the subcutaneous, perivertebral and total NAT volumes estimated by both evaluators and the inter-evaluator estimate differences of these variables.

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Study 4

Objectively measured sedentary time and physical activity are associated with neck adipose tissue volume in young sedentary adults

ABSTRACT

Background: The role of lifestyle-behaviours on neck adipose tissue (NAT), a fat depot that seems to be involved in the pathogenesis of different cardiometabolic diseases and in inflammatory status, is unknown. We examine the relationship of sedentary time and physical activity with neck adiposity in young adults.

Materials and Methods: A total of 134 young adults (69% women, 22.7±2.1 years, BMI 24.9±4.6 kg/m2) participated in this cross-sectional study. The time spent in sedentary behaviour and in PA of different intensity was objectively measured for 7 consecutive days (24 h/day), using a wrist (non-dominant)-worn accelerometer. NAT volume was assessed using positron emission tomography combined with computed tomography (PET-CT). Compartmental (subcutaneous, intermuscular and perivertebral) and total NAT volumes were determined at the level of vertebra C5. Anthropometric indicators and body composition were determined by dual energy x-ray absorptiometry.

Results: The time spent in light physical activity, moderate physical activity (MPA) and moderate-to-vigorous physical activity (MVPA) were inversely associated with the intermuscular NAT volume (r=-0.354, P=0.04; r=-0.351, P=0.04; and r=-0.354, P=0.04, respectively) in men. Accordingly, MPA, MVPA and MVPA in bouts of ≥10 min were inversely associated with total NAT volume in men (r=-0.400, P=0.03; r=-0.413, P=0.02; r=-0.429, P=0.02), and sedentary time directly related (r=0.377, P=0.036). None of these associations was observed in women (P>0.05). After adjusting for multiplicity, all results became non-significant (P>0.05).

Conclusions: These findings suggest that the specific characteristics of PA (time and intensity) might have sex-dependent implications for upper body fat mobilization, and therefore in the accumulation of NAT.

Keywords: cardiometabolic risk, neck circumference, physical activity levels, sedentary lifestyle, upper subcutaneous adipose tissue, volumetric assessment.

INTRODUCTION

The prevalence of overweight and obesity is increasing globally; approximately 1.3 billion adults are now overweight and 335 million are obese (1). Obesity is associated with cardiometabolic disease, including type 2 diabetes, hypertension, dyslipidaemia and coronary heart disease (2), and is therefore a substantial financial burden on health care systems.

Interventions designed to change lifestyle behaviours such as sedentary time and physical activity (PA) (with the aim of meeting international PA recommendations (3)) can be undertaken to combat obesity and its related comorbidities (4). Individuals who practise higher levels of PA are less likely to gain weight and show better weight maintenance (5; 6). Indeed, high levels of moderate-vigorous PA (MVPA) are related to lower abdominal and total body fat (7; 8). A growing body of evidence also suggests that sedentary time is directly associated with the classical anthropometric indicators of obesity, such as body mass index (BMI) and waist circumference, with total body fat and visceral adipose tissue (VAT) mass (6; 9).

Recent studies have highlighted the existence of previously unrecognised fat depots that may explain part of the increased cardiometabolic risk (CMR) related to obesity (10; 11). For instance, neck adipose tissue (NAT) has been shown to increase with increasing adiposity, following different accumulation patterns across the different neck compartments (subcutaneous, intermuscular and perivertebral regions) - at least in patients with certain malignant/benign tumours - a pattern that also differs between the sexes (12). Further, it has been shown that total NAT, as well as the accumulation of NAT in these compartments, correlates with CMR factors and the prevalence of metabolic syndrome (12-14). Indeed, Lee et al.(15) showed that upper body subcutaneous fat is related to CMR factors, independently of BMI, neck circumference and VAT mass. Furthermore, those subjects in the upper quartile of neck fat

volume/height, were also those who showed a higher all-cause mortality in a follow-up study, suggesting that neck adiposity is an indicator of poor long-term outcome (14). Therefore, strategies that could help modulate or prevent NAT accumulation (together with total and central body fat accumulation) are of clinical interest. The aims of the present work were to determine: i) whether the time spent in sedentary behaviour and PA is related to compartmental and total NAT volumes in a cohort of young healthy adults; and ii) whether there are differences in the compartmental and total NAT volumes between those subjects who meet/do not meet international PA recommendations.

MATERIALS AND METHODS

Study design and study subjects

A total of 134 young healthy adults (18-25 years old, 69% women) participated in the present cross-sectional study. The subjects were enrolled in the ACTIBATE – Activating brown adipose tissue through exercise - study (ClinicalTrials.gov, ID: NCT02365129) (16) via advertisements in the electronic media and physical leaflets. All subjects were non-smokers, reported themselves to be sedentary (<20 min physical activity on <3 days/week), had a stable body weight over the last 3 months (change <3 kg), and had no cardiometabolic disease (e.g., type 2 diabetes, hypertension, etc.). Assessments were performed between October and December 2015 and 2016 in Granada (Spain). The study protocol was designed in accordance with the Declaration of Helsinki (2013 revision) and approved by the Human Research Ethics Committee of the University of Granada (nº 924) and the Servicio Andaluz de Salud. All subjects signed an informed consent to be included.

Procedures

Time spent in sedentary behaviour and physical activity

The time spent in sedentary behaviour and PA were objectively measured for seven consecutive days (24 h/day), using a wrist (non-dominant)-worn GT3X+ accelerometer (ActiGraph Pensacola, FL, US) (16). Subjects were carefully explained how to wear the accelerometer, emphasizing that it should be removed only during bathing or swimming, etc. All accelerometers were initialized to store raw accelerations at a sampling frequency of 100 Hz (17); the raw acceleration data were obtained using ActiLife v.6.13.3 software (ActiGraph, Pensacola, FL, US). The raw ".csv" files were imported into R software (v.3.1.2, https://www.cran.r-project.org/) and processed using the GGIR package (v.1.5-24, https://cran.r-project.org/web/packages/GGIR/) (18). The GGIR processing

methods included: 1) auto-calibration of the data according to local gravity (19); 2) the calculation of the Euclidean Norm Minus One (ENMO) i.e., (√(x^2+ y^2+ z^2))-1G (where 1 G ~ 9.8 m/s2) with negative values rounded to zero; 3) the determination of the non-wear time (i.e., when the subjects did not wear the accelerometer) based on the raw acceleration of the three axes; briefly, each 15 min block was classified as non-wear time if the standard deviation of any two axes was <13 mG in the surrounding 60 min window, or if the values for any two axes was <50 mG; 4) the detection of sustained abnormally high accelerations, i.e., >5.5 G for at least 15 min; 5) the imputation of detected non-wear time and abnormally high accelerations using the mean of acceleration for the same recording period over days for which valid results were available; 6) the identification of the waking and sleeping hours using an automated algorithm guided by subject diary reports (20); and 7) the estimation of the time spent in sedentary behaviour and in PA of different intensity, i.e., light (LPA), moderate (MPA), vigorous (VPA), and moderate-vigorous (MVPA), using age-specific cut-points for the ENMO (21; 22). The time spent in MVPA in bouts of ≥10 min (MVPA10min) was also calculated. The mean ENMO (mg) during waking time (i.e., the number of hours that the subjects spent awake) was used as an indicator of the overall PA. For a subject's data to be included in analyses, the accelerometer had to have been worn for ≥16 h/day for at least 4 days (including at least 1 weekend day).

To examine the differences in compartmental and total NAT between subjects who met/did not meet international PA recommendations, subjects were divided into those who performed ≥150 min/week of bouted MVPA (as recommended), and those who performed <150 min/week.

The subjects also wore a hip-attached accelerometer and the above analyses were repeated using the hip-based cut-offs described by Hildebrand et al. for

classifying the hip ENMO into sedentary time - LPA, MPA, VPA and MVPA - and for determining the MVPA10min (21; 22).

18F-FDG PET/CT

Subjects arriving at the research centre confirmed that they met the following pre-established conditions: i) to be in a fasting state (at least 6 h long), ii) to have slept as usual, iii) to have refrained from any moderate or vigorous physical activity within the last 24 and 48 h respectively, iv) not to have consumed any alcoholic or stimulant beverages within the previous 6 h, or any drug that might have affected the peripheral circulation within the last 24 h. Since the original aim of the ACTIBATE study (ClinicalTrials.gov ID: NCT02365129) was to detect the volume and activity of BAT (16), participants were submitted to a personalized cooling protocol, and then they underwent a PET/CT (positron emission tomography combined with computed tomography) More specifically, after 60 min of personalized cooling, subjects were injected with a bolus of 18F-FDG (180.6 ± 5.8 MBq ≈2.9 MBq/kg), and 1 h later, subjects laid supine on a flat stretcher - with a thin pillow under the head to make them feel more comfortable. Then, subjects underwent a low dose CT scan (120 kV) using a Siemens Biograph 16 PET/CT scanner (Siemens, Erlangen, Germany) - for attenuation correction and anatomical localization purposes. Immediately after, one static acquisition of 2 PET BED positions (6 min each) was performed from the atlas vertebra to the mid chest region, using the same scanner.

Neck measurements

Quantification of neck adipose tissue

For the purpose of the main study (quantification of NAT), only the attenuation correction CT-component was used. All CT scans were analysed by JMP, using a software based on the Beth Israel plugin for FIJI http://sourceforge.net/projects/bifijiplugins/. To determine NAT volumes and

distribution across different compartments, several regions of interest (ROIs) were outlined at the level of C5 based on the procedure reported by Torriani et al. (12) (slightly modified), using a 3D-axial technique. This was so-performed to allow comparisons between studies, and because the neck circumference (NC) is normally measured at the laryngeal prominence, i.e., approximately at the level of C5. The NAT volumes in these ROIs were calculated by determining the number of pixels within the radiodensity range of -300 to -10 Hounsfield Units (HU). An extended description of how the ROIs were drawn and analysed for each NAT compartment can be found in the supplementary material, study 3. Briefly, the three main NAT compartments were defined as:

i) subcutaneous NAT: adipose tissue in the posterior neck, between the skin and the deep cervical fascia.

ii) intermuscular NAT: adipose tissue between the sternocleidomastoid, levator scapulae, semiespinalis and trapezius muscles, separated from the subcutaneous fat by the deep cervical fascia. Special attention was paid to ensure there was no overlap between the subcutaneous NAT and this compartment.

iii) perivertebral NAT: adipose tissue interspersed between the muscles surrounding vertebra C5.

Under a 3-D sagittal view, another ROI at the height of C5 was outlined to determine the total NAT (no compartment differentiation) and neck lean tissue volumes (23). This ROI was drawn parallel to the sagittal axis of the body, and included the entire neck length and width from the upper to the lower part of vertebra C5. Pixels were considered to represent NAT when they fell within the -300 to -10 HU radiodensity range, whereas those in the 9 to 150 HU range were considered to represent lean tissue (including skeletal muscle tissue, blood vessels, and some internal organs) (23). The coefficient of variation for the NAT was then calculated. Further details can be found in the supporting information

of a recent publication (submitted, reference to be supplied). Lastly, percentage NAT (%) was calculated by dividing the total NAT volume by the total neck volume x 100, and the total NAT/height ratio calculated by dividing the total NAT volume by subject height (14).

Neck circumference

NC was measured using an inextensible metallic tape over the thyroid cartilage, perpendicular to the longitudinal axis of the neck (24). For this measurement, subjects remained standing or sitting with the head in the Frankfort plane and the shoulders relaxed.

Anthropometry and body composition measurements

Weight and height were measured using a Seca model 769 calibrated digital scale and a model 213 portable stadiometer respectively (both from SECA, Hamburg, Germany). Body mass index (BMI) was calculated as body weight/squared height (kg/m2). Waist circumference was measured at the minimum perimeter, or when subjects showed abdominal obesity, in a horizontal plane above the umbilicus (24). All anthropometric measurements (except for weight and height) were assessed twice, and the mean used in analyses. Fat mass, lean body mass, and VAT mass were determined by dual X-ray absorptiometry using a Hologic Wi device (Hologic, Bedford, Massachusetts, USA).

Statistical analysis

Subject characteristics were recorded using descriptive statistics. Given the significant interactions of sedentary time and PA with sex on neck measurements, and the fact that women and men seem to have different NAT accumulation patterns (12), analyses were performed separately for women and men. All variables related to NAT were square root-transformed. Pearson correlation coefficients were initially used to examine the relationship of sedentary time/PA with compartmental and total NAT volume and neck

circumference. Partial correlations were then determined to re-examine these associations after adjusting for waking time, BMI, and percentage body fat. Adjustments for multiple comparisons were performed with the Benjamini-Hochberg procedure (False Discovery Rate-FDR- correction), to control the overall type I error rate. This procedure was applied for the main analyses. The t-test for independent sample was used to compare the compartmental and total NAT volume, and NC, of the subjects who met/did not meet international PA recommendations. All analyses were performed using the Statistical Package for the Social Sciences v.24 (IBM Inc., Chicago, IL, USA).

RESULTS

All 134 subjects had complete PA and NAT data, and all underwent 18F-FDG-PET/CT scanning to quantify their upper body adipose tissue. However, after checking the CT scans, 21 subjects (15 women and 6 men) were excluded from the analyses of the subcutaneous and intermuscular NAT, 22 (16 women and 6 men) from the analysis of the perivertebral NAT, and 25 (15 women and 10 men) from the total NAT analysis. For these subjects, all of whom had excess upper body fat (see limitations section), it was impossible to accurately outline the required ROIs.

Table 1 shows the subjects' characteristics stratified by sex. The accelerometers were worn for a mean 23.7±0.4 h/day, and showed subjects to spend 79% of their waking time (16.63±0.8 h/day) in sedentary behaviour. Significant differences were seen between women and men (all P≤0.022) in terms of perivertebral NAT, NC, and body composition.

Table 1. Descriptive characteristics of the study subjects.

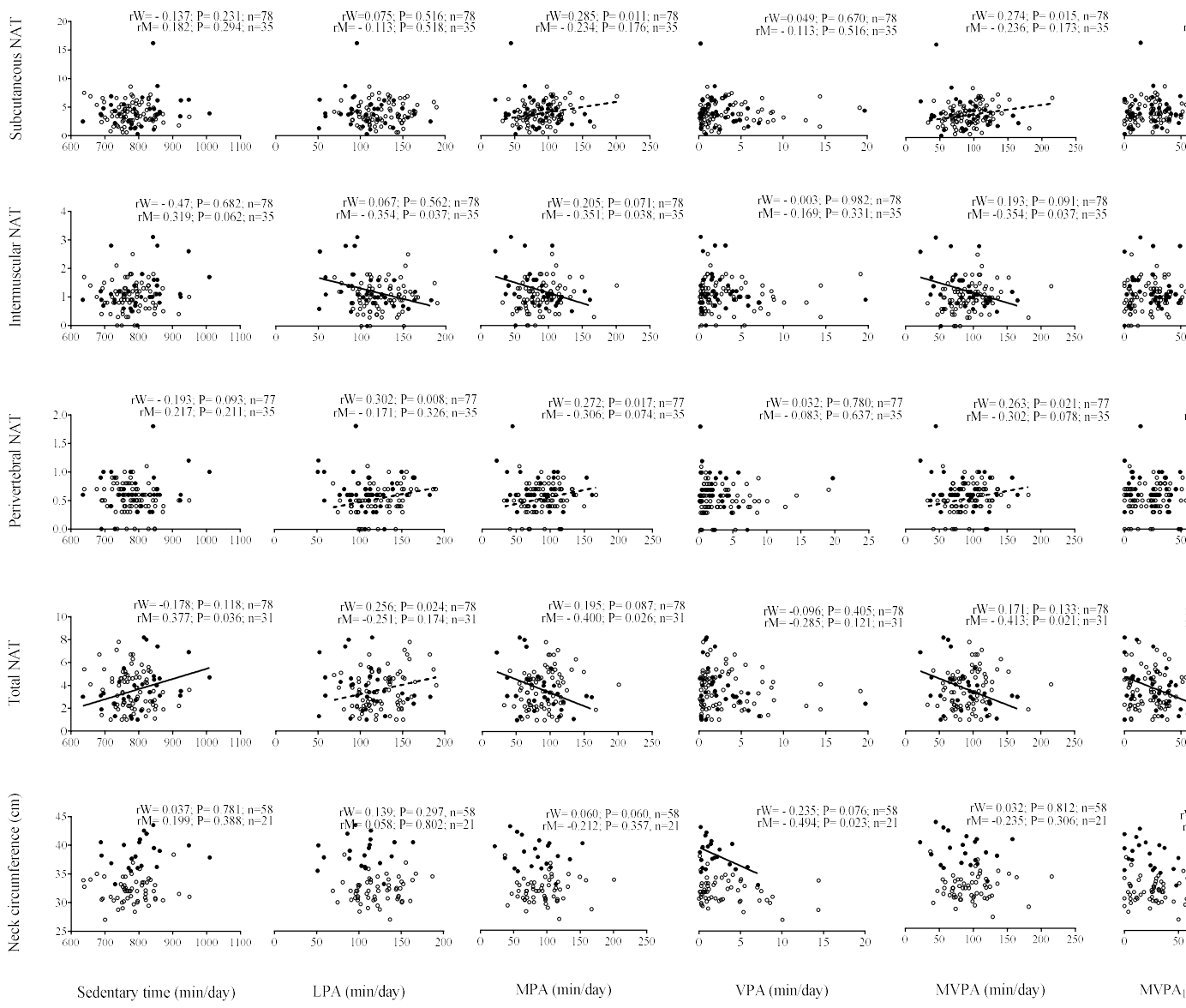
	ALL			WOMEN			MEN			P
Age (years)	22.1	(2.2)	134	22.0	(2.1)	93	22.3	(2.3)	41	0.430
Sedentary time and PA										
Accelerometry: valid measuring days	6.8	(0.5)	134	6.8	(0.5)	93	6.7	(0.5)	41	0.450
Non-wear time (hour/day)	0.3	(0.4)	134	0.3	(0.4)	93	0.4	(0.5)	41	0.125
Waking time (min/day)	998	(48)	134	997	(46)	93	1001	(53)	41	0.702
Sedentary time (min/day)	789	(65)	134	781	(58)	93	807	(78)	41	0.058
LPA (min/day)	119	(28)	134	123	(26)	93	108	(31)	41	**0.004**
MPA (min/day)	88	(30)	134	90	(29)	93	83	(32)	41	0.191
VPA (min/day)	3	(4)	134	3	(4)	93	2	(3)	41	0.338
MVPA (min/day)	91	(32)	134	93	(30)	93	85	(34)	41	0.179
$MVPA_{10min}$ (min/day)	24	(21)	134	24	(21)	93	24	(20)	41	0.989
Overall PA (mG/5s)	32	(8)	134	33	(8)	93	30	(9)	41	0.073
Neck measurements										

								Study 4	
Subcutaneous NAT (mL)	4.1	(2.2)	113	3.9	(1.8)	78	4.3 (2.8)	35	0.429
Intermuscular NAT (mL)	1.1	(0.6)	113	1.0	(0.5)	78	1.2 (0.7)	35	0.065
Perivertebral NAT (mL)	0.6	(0.3)	112	0.5	(0.2)	77	0.7 (0.3)	35	**0.022**
Total NAT (mL)	3.7	(1.7)	109	3.6	(1.6)	78	3.7 (1.9)	31	0.822
Neck circumference (cm)	33.8	(3.7)	79	32.1	(2.1)	58	38.6 (2.6)	21	**≤ 0.001**
Anthropometry and body composition									
BMI (kg/m^2)	24.9	(4.6)	134	23.8	(3.9)	93	27.2 (5.1)	41	**≤ 0.001**
Waist circumference (cm)	80.9	(14.0)	131	76.3	(10.8)	90	91.1 (15.0)	41	**≤ 0.001**
Lean mass (kg)	41.5	(9.5)	134	36.4	(5.0)	93	53.1 (6.5)	41	**≤ 0.001**
Fat mass (kg)	25.1	(8.9)	134	24.7	(7.8)	93	26.3 (11.1)	41	0.388
Body fat percentage (%)	36.0	(7.3)	134	38.3	(6.0)	93	30.9 (7.5)	41	**≤ 0.001**
VAT mass (g)	340.1	(179.0)	134	297.0	(163.7)	93	438.1 (175.3)	41	**≤ 0.001**

Mean values (standard deviation) and sample sizes are provided. The t-test for independent samples was used to compare the results for women and men. All the variables related to NAT were square root-transformed to render their distributions closer to normal. BMI: Body mass index; LPA: light physical activity, MPA: moderate physical activity, MVPA: moderate-vigorous physical activity, $MVPA_{10min}$: moderate-vigorous physical activity in bouts of 10 minutes, NAT: Neck adipose tissue; VAT: visceral adipose tissue, VPA: vigorous physical activity.

Figure 1 shows the bivariate association between time spent in sedentary behaviour/PA and the compartmental and total NAT volumes in women (rW) and men (rM). In the women, LPA was directly associated with the perivertebral and total NAT volumes (r=0.302, P=0.008 and r=0.256, P=0.024 respectively), and MPA and MVPA were directly associated with the subcutaneous (r=0.285, P=0.011 and r=0.274, P=0.015 respectively) and perivertebral NAT (r=0.272, P=0.017 and r=0.263, P=0.021 respectively) volumes. In the men, the LPA, MPA and MVPA were inversely related to the intermuscular NAT volume (r=-0.354, P=0.037, r=-0.351, P=0.038, r=-0.354, P=0.037 respectively), and MPA, MVPA and MVPA10min with the total NAT volume (r=-0.400, P=0.026; r=-0.413, P=0.021, r=-0.429, P= 0.016). The VPA also showed an inverse relationship with NC (r=-0.494, P=0.023). In addition, sedentary time was positively associated with total NAT volume (r=0.377, P=0.036).

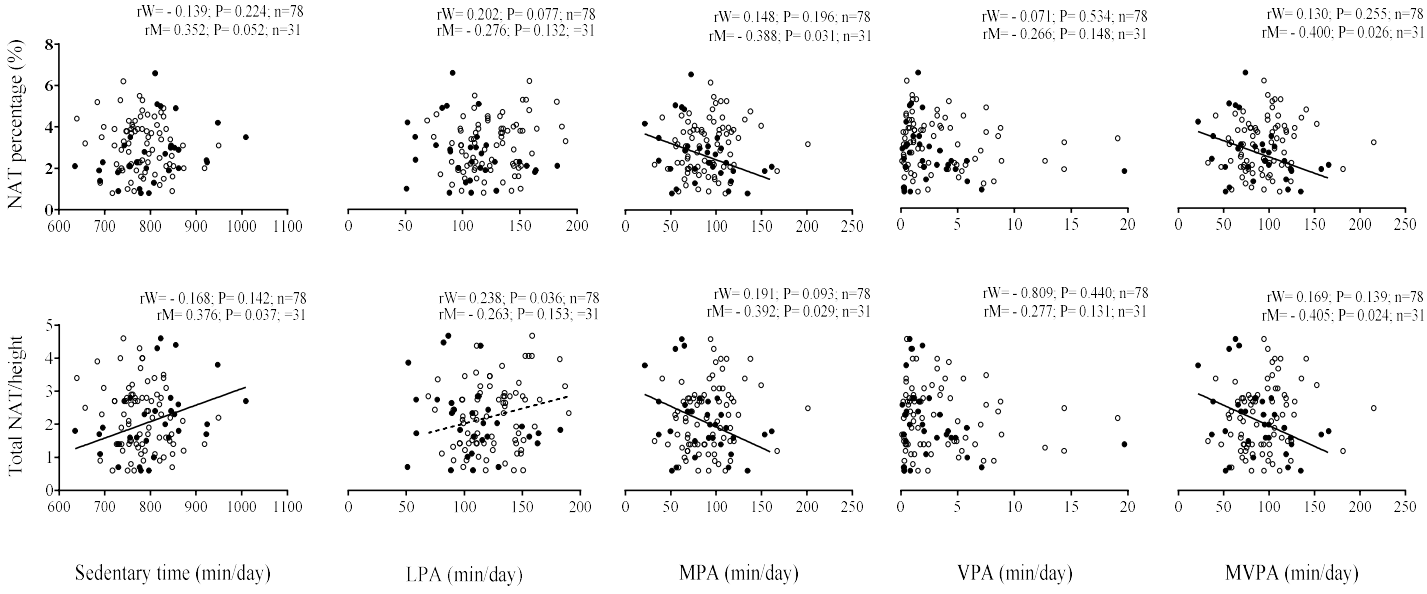
Figure 1. Associations between objectively measured sedentary time/physical activity and compartmental and total NAT volum



Pearson correlation coefficients were determined to examine the association of objectively measured sedentary time/physical activity and compartmental and total NAT volumes and neck circumference. P-values and sample sizes are provided for women (rW) and for men (rM). Values for all variables relating to NAT were square root-transformed. Of note, after adjusting for multiplicity, all results became non-significant (all P>0.05). LPA: light physical activity, MPA: moderate physical activity, MVPA: moderate-vigorous physical activity, MVPA10min: moderate-vigorous physical activity in bouts of 10 minutes, NAT: Neck adipose tissue; NC: Neck circumference, VPA: vigorous physical activity.

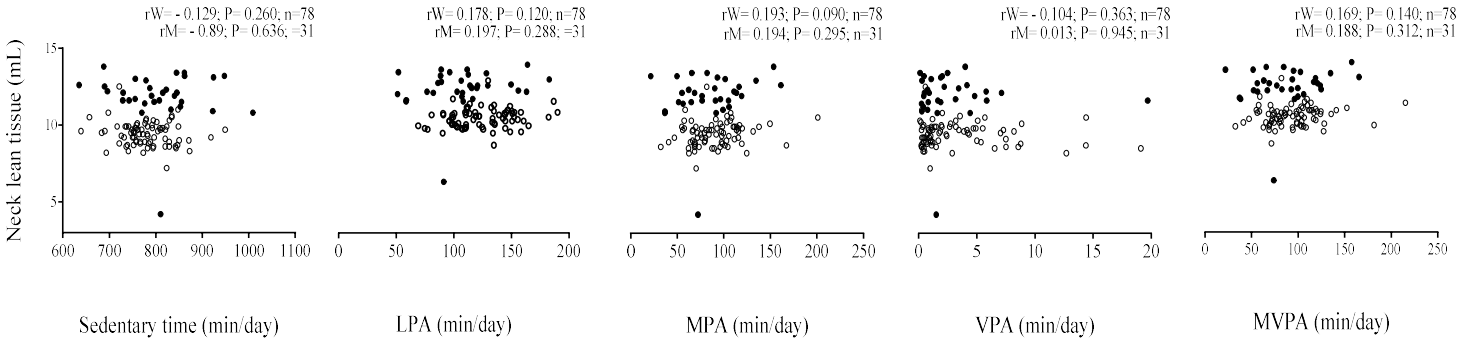
Similar results were obtained when the relationships between sedentary time and physical activity and NAT percentage and total NAT/height in men were examined, although a direct relationship between LPA and total NAT/height was only seen in the women (P=0.037) (Figure 2). No association was seen between sedentary time/PA and neck lean tissue volume (all P>0.05, see Figure 3).

Figure 2. Association of objectively measured sedentary time and physical activity with NAT percentage (%) and total NAT/hei



Pearson correlation coefficient, P-value and sample size are provided for women (rW) and for men (rM). LPA: light physical ac
activity, MVPA: moderate-vigorous physical activity, MVPA10min: moderate-vigorous physical activity in bouts of 10 minutes,
Visceral adipose tissue, VPA: vigorous physical activity.

Figure 3. Association of objectively measured sedentary time and physical activity with neck lean tissue volume.



Pearson correlation coefficient, P-value and sample size are provided for women (rW) and for men (rM). LPA: light physical ac
activity, MVPA: moderate-vigorous physical activity, $MVPA_{10min}$: moderate-vigorous physical activity in bouts of 10 minutes, VA
vigorous physical activity.

Re-examination of the relationship between sedentary time/PA and neck measurements after adjusting
returned similar results for the women, except for when adjusting for BMI (see Table 2).

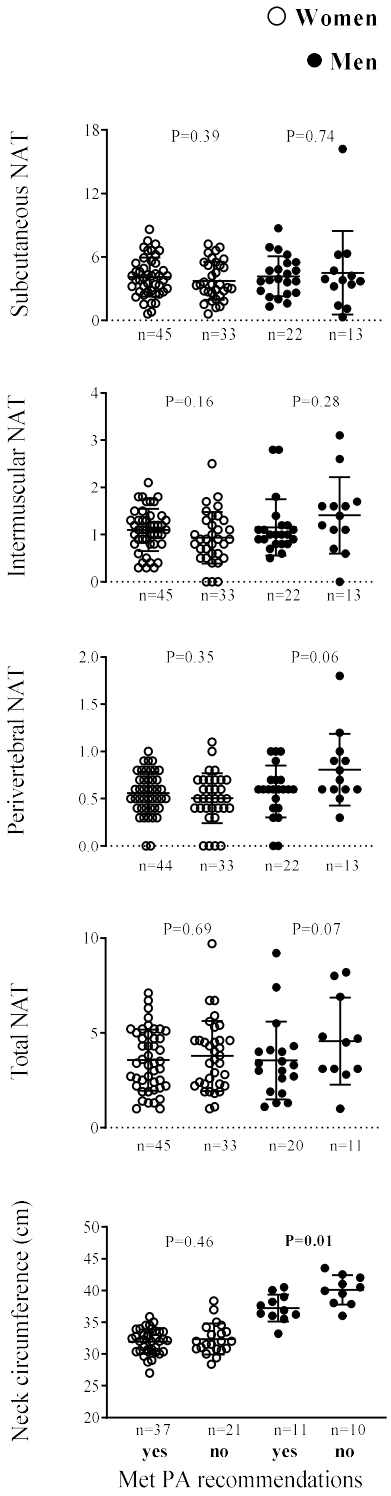
Table 2. Association between objectively measured sedentary time/physical activity and compartmental and total NAT volume adjusting for potential confounders.

	WOMEN									
	Sedentary time (min/day)	LPA (min/day)	MPA (min/day)	VPA (min/day)	MVPA (min/day)	$MVPA_{10min}$ (min/day)	Overall PA (mG/5s)	Sedentary time (min/day)	LPA (min/day)	MPA (min/day)
Model 1 (adjusted for waking time)										
Subcutaneous NAT	-0.21	0.06	**0.28***	0.04	**0.27***	0.14	0.20	0.19	-0.12	-0.23
Intermuscular NAT	-0.13	0.03	0.19	-0.01	0.18	0.09	0.11	**0.38***	**-0.37***	**-0.35***
Perivertebral NAT	**-0.30****	**0.29***	**0.26***	0.02	**0.25***	0.15	0.22	0.26	-0.18	-0.31
Total NAT	**-0.25***	**0.26***	0.19	-0.10	0.17	-0.08	0.13	**0.37***	-0.27	**-0.40***
Neck circumference (cm)	-0.06	0.10	0.04	-0.25	0.01	-0.07	-0.05	0.11	0.03	-0.21
Model 2 (adjusted for BMI)										
Subcutaneous NAT	-0.06	-0.10	0.11	0.06	0.11	0.05	0.08	0.03	-0.07	-0.11
Intermuscular NAT	0.07	-0.13	-0.02	0.00	-0.02	-0.02	-0.05	0.21	**-0.46****	-0.29
Perivertebral NAT	-0.15	**0.23***	0.16	0.04	0.15	0.10	0.14	0.10	-0.14	-0.22
Total NAT	-0.09	0.10	-0.06	-0.15	-0.07	**-0.23***	-0.05	0.30	**-0.40***	**-0.41***
Neck circumference (cm)	0.19	-0.04	-0.19	**-0.32***	-0.22	-0.15	**-0.26***	-0.12	0.34	0.16
Model 3 (adjusted for percentage body fat)										
Subcutaneous NAT	-0.16	0.12	**0.33****	0.08	**0.32****	0.19	**0.26***	-0.06	0.04	-0.05
Intermuscular NAT	-0.05	0.11	**0.23***	0.02	0.22	0.14	0.16	0.03	-0.25	-0.15
Perivertebral NAT	-0.20	**0.33****	**0.27***	0.04	**0.26***	0.17	**0.24***	-0.05	-0.02	-0.12
Total NAT	-0.19	**0.30****	0.20	-0.15	0.17	-0.08	0.14	0.04	-0.12	-0.23
Neck circumference (cm)	0.07	0.12	0.03	-0.24	0.01	-0.04	-0.07	-0.21	0.41	0.18

Partial correlations were determined to examine the association between sedentary time/physical activity and compartmental and total NAT volumes and neck circumference after adjusting for waking time (model 1), BMI (model 2), and body fat percentage (model 3). The total number of women and men (respectively) included in each analysis was as follows: subcutaneous and intermuscular NAT (78 and 35), perivertebral NAT (77 and 35), total NAT (78 and 31), and neck circumference (58 and 21). Values for all variables relating to neck adipose tissue were square root-transformed to render their distributions closer to normal. Partial correlation coefficients and P-value are provided. Significant differences are shown in bold: *P≤0.05, **P≤0.01, ***P≤0.001. LPA: light physical activity, MPA: moderate physical activity, MVPA: moderate-vigorous physical activity, MVPA10min: moderate-vigorous physical activity in bouts of 10 minutes, NAT: neck adipose tissue, VPA: vigorous physical activity.

In men, the results remained similar when adjusting for waking time. However, the noted associations were attenuated when adjustment was made for BMI, and most associations disappeared after adjusting for percentage body fat (see Table 2). We also repeated the analyses adjusting for energy intake, and only the associations between NC and VPA in men changed becoming non-significant (P >0.05, data not shown). In addition, no significant differences (P>0.05) were seen in the compartmental and total NAT volumes between subjects who met/did not meet the international PA recommendations - although those men who met them showed a trend towards having smaller perivertebral and total NAT volumes (P=0.06 and 0.07, respectively; Figure 4). Regarding the neck circumference, those men who met the PA recommendations had lower values than those who did not meet them (P=0.01).

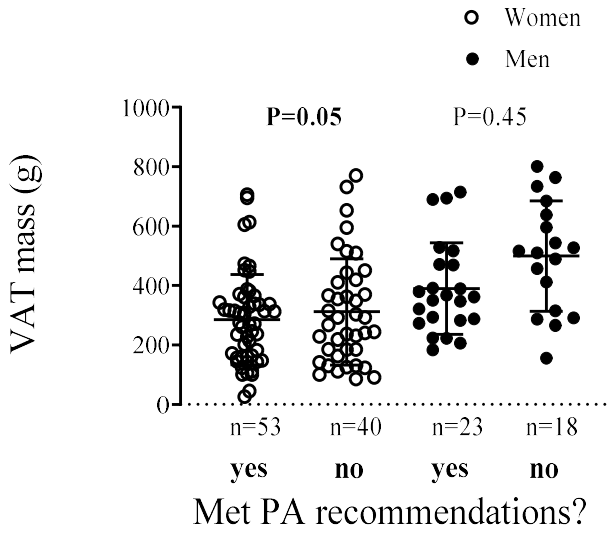
Figure 4. Comparison of compartmental and total NAT volumes and neck circumference for groups of participants who met/did not meet international PA recommendations.



Mean values, standard deviations and P-value (t-test for independent samples) are provided for women and men. Values for all variables relating to neck adipose tissue were square root-transformed to render their distributions closer to normal. NAT: neck adipose tissue, NC: neck circumference, PA: physical activity.

When comparing VAT mass between these groups, women who met the PA recommendations also seemed to have lower values than those who did not meet them (P=0.05; Figure 5).

Figure 5. Comparison of visceral adipose tissue (VAT) mass between groups of participants who met or not the international PA recommendations.



Independent Samples T-test were performed. P-value and sample size are provided for women and men. Statistically significant values are indicated in bold. Yes = comply with the daily recommendations of PA, not: not comply with the daily recommendations of PA.

Of note is that after adjusting for multiple comparisons, all results related to the main analyses without ad

Figure 1), became non-significant (all P>0.05, data not shown).

Table 3 shows the relationships detected between sedentary time/PA categories and anthropometric and b

indicators.

Table 3. Association of objectively measured sedentary time and physical activity with anthropometric and body co

	WOMEN										
	Sedentary time (min/day)	LPA (min/day)	MPA (min/day)	VPA (min/day)	MVPA (min/day)	$MVPA_{10min}$ (min/day)	Overall PA (mG/5s)	Sedentary time (min/day)	LPA (min/day)	MPA (min/day)	(
BMI (kg/m²)	-0.15	**0.25***	**0.28****	-0.01	**0.27****	0.07	**0.21***	**0.31***	-0.15	**-0.35***	
Waist circumference (cm)	-0.12	0.16	**0.25***	-0.05	**0.23***	0.13	0.16	**0.36***	-0.11	**-0.40***	
Waist-Hip ratio	0.05	0.02	0.05	-0.11	0.04	0.01	-0.01	0.27	-0.13	-0.26	
Lean mass (kg)	**-0.21***	**0.34*****	**0.34*****	0.02	**0.32*****	0.19	**0.29****	0.07	0.08	-0.12	
Fat mass (kg)	-0.14	**0.21***	0.20	-0.04	0.19	0.01	0.15	**0.33***	-0.20	**-0.37***	
Body fat percentage (%)	-0.06	0.07	0.07	-0.04	0.06	-0.06	0.04	**0.41****	-0.25	**-0.40****	
VAT mass (g)	-0.11	**0.26***	0.15	-0.08	0.13	-0.08	0.10	0.27	-0.12	-0.23	

Pearson correlation coefficient is provided for women and men. A total of 93 women and 41 men were included in the analys
waist circumference and waist-hip ratio, in which only 90 women were included. Statistically significant values are indica
***P≤0.001. BMI: Body mass index; LPA: light physical activity, MPA: moderate physical activity, MVPA: moderate-vigorou
moderate-vigorous physical activity in bouts of 10 minutes, PA: physical activity, VPA: vigorous physical activity, VAT: viscera

Sensitivity analyses

Tests were performed to determine whether the relationship between sedentary time/PA and the compartmental and total NAT volumes varied after taking into account all the subjects who were originally excluded. Overall, the relationship between the PA categories and subcutaneous and total NAT volumes disappeared in the women ($P>0.05$). However, the results remained similar for the men (data not show).

One male subject - an obese man with the largest NC recorded - had compartmental NAT volumes that exceeded those of the remaining subjects (subcutaneous NAT: 16.2 mL, z score = 3.58; intermuscular NAT: 3.1 mL, z score=2.61; and perivertebral NAT: 1.8 mL, z score= 3.92). Since these values are biologically plausible, and the participant had a total NAT volume within the normal range, all analyses were performed again excluding this subject. The results remained unchanged (data not show).

When we repeated the analyses using the hip-attached accelerometer data, results slightly differed - especially in women (Supplementary Material, table S1). In addition, the differences in compartmental and total NAT between subjects who met/did not meet international PA recommendations using the hip-based cutoffs are shown in table S2.

Coefficients of variation (CVs) of NAT measures (indicating the consistency of our data), and Bland-Altman plots comparing inter-evaluator estimate differences in NAT assessment, are also provided in the Supplementary Material.

DISCUSSION

The present results show that less sedentary time and more time spent in PA are associated with lower compartmental and total NAT volumes in men. However, in women the opposite was seen, with several PA categories directly associated with the compartmental and total NAT volumes. These findings suggest that the association between sedentary time/PA and compartmental and total NAT may be heterogeneous and sex-dependent, although the results should be interpreted with caution given the relatively small number of males included, and that associations became non-significant after adjusting for multiplicity. This provides new insight that might be useful in the search for strategies to help modulate or prevent NAT accumulation along with that of total and central body fat.

The literature contains little information with which to compare the present findings. Previous experiments have shown upper body fat to be a major contributor to the available systemic free fatty acids (even more than central fat) (12; 25; 26)- a consequence of the direct access of free fatty acids (FFA) to the liver through the portal system (27; 28). In vitro studies (29-31) have also shown that upper body adipocytes isolated from lean people and those with obesity are more responsive to lipolytic adrenergic stimulation than lower-body adipocytes, suggesting that upper body fat depots have a greater propensity to mobilize fatty acids. In this context, it might be hypothesized that NAT, an upper body fat depot, is a major contributor of systemic FFAs, especially during exercise or other PA when FFAs are mobilized to satisfy skeletal muscle energy demands. Since previous studies have shown the accumulation of NAT to be a predictor of CMR, a more pro-inflammatory profile, and increased long-term mortality (12; 14), it is important to understand whether PA can be used to prevent or reduce NAT accumulation.

In the present work it was hypothesized that women and men who spent less time in sedentary behaviour, and more time at different intensities of PA, might also be those with smaller compartmental and total NAT volumes. Interestingly, the results in men were as expected, but in the women a direct relationship was seen between certain PA categories and compartmental and total NAT volumes. The lack of experimental and epidemiological evidence examining sex differences in the interaction between exercise and metabolism and its underlying mechanisms hampers the understanding of the present results. However, gender greatly determines fat metabolism; for example women and men show differences in fat oxidation, regional fat deposition and lipolysis rates (32; 33). In fact, at the whole body level it has been suggested that, compared to men, women show greater fatty acid mobilization during low and moderate intensity exercise (although these differences might be partially attributed to the larger fat stores of women) (34). Further, under conditions of energy restriction (35) and/or when PA-associated energy expenditure is increased, women and men seem to mobilize fatty acids from different depots and at different rates, revealing important local differences in fat metabolism (36). For instance, women better mobilize fatty acids from the subcutaneous abdominal body fat (due to their greater expression of beta-adrenergic receptors, and the lesser sensitivity of alpha-adrenergic receptors), but do so more poorly from the lower-body depots (gluteal and femoral adipose tissue) (29; 37). In contrast, men show higher beta-adrenergic and lower alpha-adrenergic receptor sensitivity in the visceral adipose tissue, and mobilize more FFAs from this depot (32; 37). It might therefore be speculated that the opposite trends observed in the relationship between PA and NAT accumulation in women and men can be explained by differences in fatty acid mobilization from the NAT. However, these results might also be explained by not having taken into account certain potential confounders, e.g., movement patterns not detected by the accelerometers, economic and social status, energy intake and diet, etc. Further, similar sex

patterns were observed in the associations between certain anthropometric and body composition variables and sedentary time/PA, e.g., those women with higher LPA, MPA and MVPA values had higher BMIs, which seems counterintuitive (see Table S1). Other lifestyle-related factors beyond sedentary time and PA might have influenced the presents results; for example, a higher PA might also be related to a higher energy intake, and therefore greater NAT accumulation. In addition, it is important to note that men normally have a higher relative lean body mass, and store less total fat than women, which might lead to larger triglyceride breakdown from ectopic fat depots during PA, favouring the reduction of adipose tissue in men's upper body depots (such as NAT).

Beyond this sexual dimorphism, it is noteworthy that sedentary time and PA were associated with the subcutaneous and perivertebral NAT volume in women, and the intermuscular NAT volume in men. This might suggest that NAT depots possess different physiological mechanisms that regulate the rate of lipolysis, sensitivity to adrenergic stimulation, and lipid storage; this has previously been suggested for other adipose tissue depots (32; 33). Taking everything together, it would seem that reducing sedentary time and increasing the time spent in PA at different intensities might help reduce NAT accumulation in men, but further studies are needed to understand the controversial results obtained for the present women.

The present results also show that, in men, VPA is inversely related to NC, and that those male participants who met the international PA recommendations had a smaller NC than those who failed to meet it. A cross-sectional study involving Latin American subjects from eight countries recently provided the first evidence that MVPA is related to NC, independent of sex, age, socioeconomic status, or level of education (38). However, this study only involved hip-worn accelerometers. Similarly, Hargens et al. (39) showed that overweight-to-obese

individuals with a higher NC undertake significantly less overall PA, independent of BMI and total body adiposity. Hence, it would seem that those subjects with higher PA levels are also those who have a lower NC. It should be remembered that NC has been suggested as a proxy of NAT accumulation in women and men (10), which further supports the idea that increasing PA might offer a means of preventing NAT accumulation in men.

The present study suffers from a number of limitations. Its cross-sectional design precludes the establishment of causality, and the difference in the size between the female and male cohorts might have exercised some influence on the results. Neither are the results automatically generalizable to non-healthy or older populations, or to people who have excess upper body fat. In those subjects with excess upper body fat it was hard to accurately outline the ROIs for distinguishing between the specific NAT compartments - an effect of the flattening and distortion of the neck compartments in the CT image, or because the jowl was included in the drawn ROI (a consequence of the position of the subjects during the PET/CT scan). CT scanning at higher resolution might have partially avoided these problems. Participants also underwent personalized cold exposure prior to the PET/CT scan, and this might have affected the radiodensity of the NAT, such that voxels initially classified as adipose tissue (i.e., within -10 to -300 HU) might fall within the radiodensity range of other tissues. However, this is highly unlikely since cold exposure induces a mean change of only ~3 HU (40).

In conclusion, the present study shows that the time spent in sedentary behaviour and PA of different intensity is associated with the compartmental and total NAT volumes in a sex-dependent manner. Interestingly, those men who spent less time in sedentary behaviour and more in PA were also those with smaller compartmental and total NAT volumes, although this might be influenced by the relatively small number of men included in the analyses. The present results

should be interpreted with caution since all associations became non-significant after adjusting for multiplicity.

Future studies should aim to examine how genetic and hormonal factors, and other lifestyle behaviours, might be related to specific sex patterns of NAT accumulation. In addition, intervention studies should be undertaken to examine whether exercise can be used to reduce NAT accumulation, and to improve CMR and the inflammatory profile. Finally, understanding how the molecular signature of specific NAT compartments changes during exercise intervention, and how this relates to CMR and the inflammatory profile, will provide insight into the mechanisms underlying the therapeutic effects of exercise.

SUPLEMMENTARY MATHERIAL

Table S1. Association of the time spent in sedentary behaviour/PA estimated by the hip-worn accelerometer with neck measure

	WOMEN				
	Sedentary time (min/day)	LPA (min/day)	MPA (min/day)	VPA (min/day)	MVPA (min/day)
Model crude					
Subcutaneous NAT	-0.048	0.100	0.104	0.072	0.107
Intermuscular NAT	-0.028	0.083	0.151	0.043	0.149
Perivertebral NAT	-0.066	0.212	0.144	-0.027	0.139
Total NAT	0.032	0.160	-0.045	-0.054	-0.049
Neck lean tissue	-0.045	0.097	0.161	0.176	0.173
Neck circumference (cm)	0.083	0.068	0.010	0.025	0.012
VAT mass (g)	0.037	0.184	0.013	-0.041	0.008
Model 1 (adjusted for waking time)					
Subcutaneous NAT	-0.148	0.087	0.094	0.067	0.097
Intermuscular NAT	-0.176	0.052	0.133	0.033	0.130
Perivertebral NAT	-0.223	0.193	0.128	-0.037	0.122
Total NAT	-0.003	0.154	-0.054	-0.059	-0.059
Neck lean tissue	-0.143	0.083	0.154	0.172	0.166
Neck circumference (cm)	-0.051	0.033	-0.011	0.013	-0.009
VAT mass (g)	-0.064	0.166	-0.001	-0.050	-0.006
Model 2 (adjusted for BMI)					
Subcutaneous NAT	-0.044	-0.079	-0.027	-0.055	-0.031
Intermuscular NAT	-0.017	-0.117	0.030	-0.105	0.017
Perivertebral NAT	-0.063	0.120	0.069	-0.118	0.057

Total NAT	0.040	-0.010	**-0.193***	-0.238	-0.211
Neck lean tissue	-0.047	0.062	0.145	0.155	0.155
Neck circumference (cm)	0.142	-0.149	-0.121	-0.086	-0.124
VAT mass (g)	0.113	-0.050	-0.195	**-0.274****	**-0.215***
Model 3 (adjusted for body fat percentage)					
Subcutaneous NAT	-0.043	0.075	0.148	0.095	0.151
Intermuscular NAT	-0.016	0.051	0.221	0.061	0.218
Perivertebral NAT	-0.062	0.201	0.161	-0.028	0.154
Total NAT	0.067	0.100	-0.022	-0.050	-0.027
Neck lean tissue	-0.047	0.110	0.158	0.174	0.170
Neck circumference (cm)	0.113	0.003	0.015	0.045	0.019
VAT mass (g)	0.112	0.105	0.031	-0.021	0.028

Continuation Table S1. Association of the time spent in sedentary behaviour/PA estimated by the hip-worn accelerometer with mass.

	MEN				
	Sedentary time (min/day)	LPA (min/day)	MPA (min/day)	VPA (min/day)	MVPA (min/day)
Model crude					
Subcutaneous NAT	0.195	-0.141	**-0.334***	-0.276	**-0.347***
Intermuscular NAT	0.244	**-0.335***	**-0.439****	-0.289	**-0.45****
Perivertebral NAT	0.263	-0.188	**-0.489****	-0.144	**-0.485****
Total NAT	**0.423***	-0.284	**-0.499****	-0.348	**-0.513****
Neck lean tissue	0.127	0.129	0.039	0.075	0.044

Neck circumference (cm)	0.420	-0.196	-0.409	-0.371	-0.426
VAT mass (g)	**0.355***	-0.256	**-0.337***	**-0.355***	**-0.355***
Model 1 (adjusted for waking time)					
Subcutaneous NAT	0.275	-0.154	-0.330	-0.283	**-0.343***
Intermuscular NAT	**0.386***	**-0.348***	**-0.439****	-0.295	**-0.45****
Perivertebral NAT	**0.437***	-0.196	**-0.492****	-0.148	**-0.486****
Total NAT	**0.532***	-0.313	**-0.489****	**-0.377***	**-0.505****
Neck lean tissue	0.070	0.115	0.053	0.062	0.057
Neck circumference (cm)	**0.49***	-0.232	-0.390	-0.396	-0.410
VAT mass (g)	**0.375***	-0.293	**-0.317***	**-0.379***	**-0.337***
Model 2 (adjusted for BMI)					
Subcutaneous NAT	-0.006	-0.030	-0.124	-0.155	-0.135
Intermuscular NAT	0.009	-0.321	-0.254	-0.158	-0.261
Perivertebral NAT	0.113	-0.104	**-0.36***	0.000	**-0.349***
Total NAT	0.185	**-0.385***	-0.334	-0.308	-0.353
Neck lean tissue	0.063	0.146	0.120	0.118	0.127
Neck circumference (cm)	0.226	0.068	-0.034	-0.178	-0.050
VAT mass (g)	0.078	-0.027	0.175	-0.139	0.158
Model 3 (adjusted for body fat percentage)					
Subcutaneous NAT	-0.030	0.042	-0.120	-0.150	-0.131
Intermuscular NAT	-0.066	-0.164	-0.195	-0.128	-0.201
Perivertebral NAT	0.033	0.004	-0.311	0.036	-0.299
Total NAT	0.071	-0.136	-0.236	-0.279	-0.257
Neck lean tissue	0.131	0.139	0.056	0.083	0.062
Neck circumference (cm)	0.164	0.147	-0.088	-0.154	-0.100
VAT mass (g)	0.000	0.103	0.113	-0.101	0.102

Pearson correlation coefficient is provided for women and men. All the variables related to NAT were transformed (square root
make its distribution closer to the normal distribution.Statistically significant values are indicated in bold: *P≤0.05, **P≤0.01, ***P
activity, MPA: moderate physical activity, MVPA: moderate-vigorous physical activity, MVPA10min: moderate-vigorous physi
minutes, NAT: neck adipose tissue, PA: physical activity, VPA: vigorous physical activity, VAT: visceral adipose tissue.

Table S2. Comparison of compartmental and total NAT volume and neck circumference across groups of participants who met
recommendations using the hip-attached accelerometer data. Mean values, standard deviation and P-value are provided for wo

	WOMEN							**MEN**				
	YES			NO			P	YES				
	N	Mean	DS	N	Mean	DS	NS	N	Mean	DS	N	Mea
Subcutaneous NAT	38	3.87	1.74	40	4.020	1.91	NS	14	3.93	1.84	21	4.54
Intermuscular NAT	38	1.07	0.43	40	0.99	0.57	NS	14	1.09	0.58	21	1.34
Perivertebral NAT	37	0.55	0.23	40	0.52	0.25	NS	14	0.54	0.33	21	0.74
Neck muscle mass	38	3.41	1.53	40	3.86	1.74	NS	13	2.70	1.12	18	4.46
Total NAT	38	9.40	0.59	40	9.45	0.94	NS	13	11.41	2.26	18	12.2
NC (cm)	34	31.89	1.96	24	32.37	2.33	NS	7	36.81	2.29	14	39.4
VAT mass (g)	46	277.30	146.97	47	316.23	178.13	NS	15	384.75	154.12	26	468.8

Mean values, standard deviation and P-value are provided for women and men. An independent Samples t-Test was performed
NAT were transformed (square root transformation) in order to make its distribution closer to the normal distribution. Yes = the
daily recommendations of PA, not: the participants did not comply with the daily recommendations of PA, NAT: neck adipose
NS: non- significant, P: value, PA: physical activity.

Data consistency and inter-evaluator estimate differences

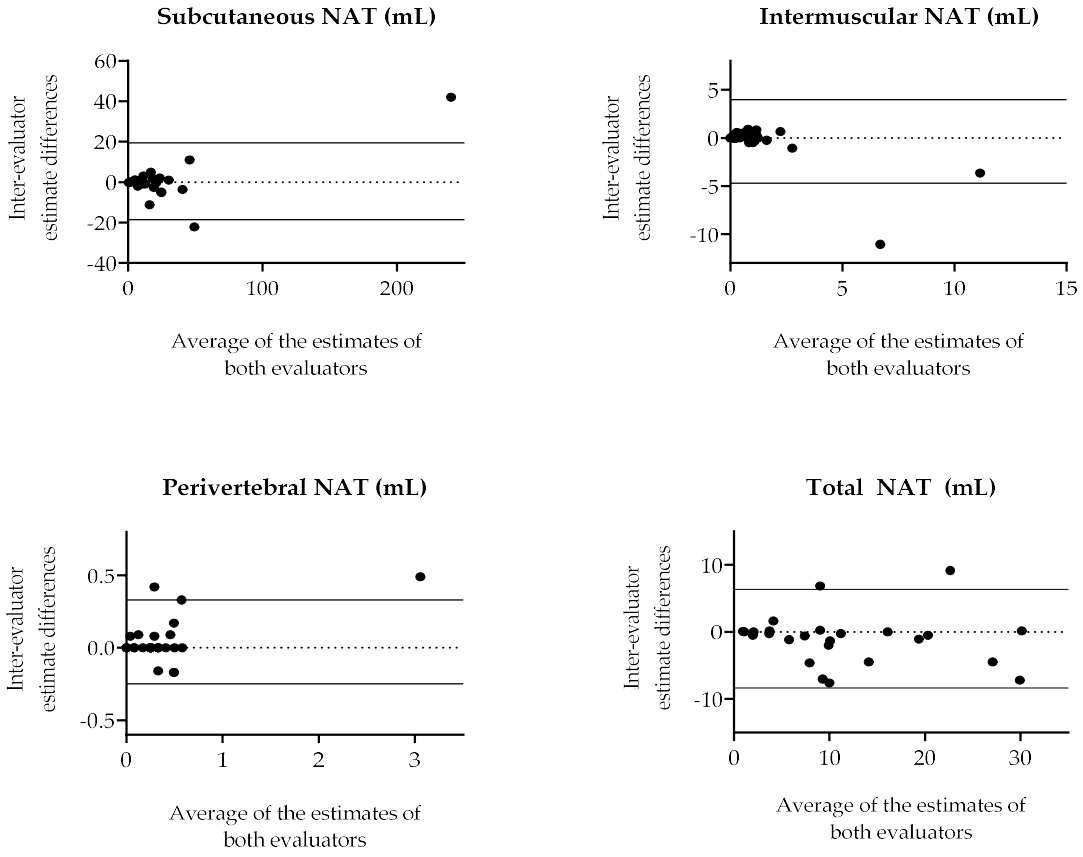
Coefficient of Variation (CV)

To examine how consistent (i.e., the uniformity in the values of the data/distribution with respect the mean) was the data related to NAT, we estimated the CV for the compartmental and total NAT volumes. The CV for NAT measures was as follow: subcutaneous (130.7%), intermuscular (107.6%), perivertebral (99.5%), and total (90.9%). Of note, as explained in the manuscript, there was an outlier participant who presented larger compartmental NAT volumes with respect their counterparts. When this participant was excluded from the analyses, the CV for the compartmental NAT measures was as follow: subcutaneous (84.5%), intermuscular (100.7%), and perivertebral (77.1%).

As can be observed the consistency of our data seems to be low, which might be explained by a high biological variability in NAT accumulation and distribution, or by methodological issues. Interestingly, the NAT measures with the lowest CV were the perivertebral and the total NAT volumes, which seems coherent, since the former variable was delineated with the smallest ROI, and the latter with a fixed ROI (i.e., both are likely to diminish the variability of the measure). Nonetheless, the subcutaneous and intermuscular NAT volumes, whose ROIs are more difficult to draw due to the CT scan resolution, the difficulties to identify certain anatomical spots, and the potential overlapping of both compartments, presented the highest CVs. This information is valuable and should be consider when interpreting the current results. Future methodological studies should aim to find accurate protocols/methods to analyse NAT.

To understand the comparability of the results when NAT volumes are estimated by different evaluators, we performed Bland-Altman plots of these variables in a subgroup of participants from the present cohort. Two researchers

independently analysed the PET/CT images of several participants to estimate compartmental NAT (n=30) and total NAT (n=26). Then, the average of the estimates obtained by both evaluators was plotted against the inter-evaluator estimate differences in NAT volumes assessment, and the limits of agreements were calculated.



As can be observed, in general terms there was a good agreement between NAT estimates performed by different evaluators, although some bias could be observed, which was mainly explained for the above mentioned outlier. In addition, few points surpassed the limits of agreement. Whether discrepancies in NAT estimates between evaluators are clinically relevant is difficult to know

given the still immature body of evidence in this field. No significant differences ($P<0.05$) were observed when the mean of NAT measures obtained by both evaluators was compared. When the outlier participant was excluded from the analysis, no relationship was found ($P<0.05$) between the average of the subcutaneous, perivertebral and total NAT volumes estimated by both evaluators and the inter-evaluator estimate differences of these variables.

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Study 5

Dose-response effect of a 6-month concurrent exercise intervention on neck adipose tissue of young adults: a randomized controlled trial

ABSTRACT

Background: Previously, our findings have shown that an increase in NAT volume and neck circumference are associated with a higher cardiometabolic risk (CMR) and a more pro inflammatory state in young, healthy, sedentary adults, independently of body mass index (BMI), body fat percentage and/or visceral adipose tissue (VAT) mass. However, if an exercise program can have an effect on neck depots is unknown.

Aim: To examine the dose-response effect of 6-month concurrent exercise intervention on the neck adipose tissue and circumference of young healthy adults.

Materials and methods: A sample size of 194 sedentary young Spanish adults between ages 18 to 25 years old was enrolled in three exercise groups: control (n=64), moderate(n=52) and vigorous-intensity (n=56) groups in ACTIBATE project (Clinical Trial Registration: NCT02365129). The NAT was estimated using positron emission tomography combined with computed tomography (PET-CT). Compartmental (Subcutaneous, Intermuscular and Perivertebral) and total NAT volumes were determined at the level of vertebra C5 and neck circumference was measured above the thyroid cartilage and perpendicular to the longitudinal axis of the neck. Dual energy x-ray absorptiometry was used to determine fat mass, lean mass, and VAT mass.

Results: Our findings show, for the first time, a small effect of a 6-month exercise intervention on neck circumference in young healthy Spanish adults who attended more than 70% of total training sessions and adhered to the training intensity established for each group in the ACTIBATE study. Specifically, the moderate and vigorous intensity groups decreased in 1.01 and 0.77 centimetres of neck circumference, respectively. Changes of NAT compartments only were observed when two low exigent filters for compliance session exercise were used.

Conclusion: An exercise intervention of 6 months of moderate and vigorous intensity can slightly reduce the neck circumference in young healthy adults. These findings are of great interest because they could explain the relationship between high intensity exercise with lipolysis of upper body fat. However, due to a little sample size and a relatively low number of men included the study, these findings must be taken with caution. Lastly, the effect of this reduction must be re-examined to improve CMR and the inflammatory profile.

Keywords: adipose tissue, neck fat accumulation, upper subcutaneous fat mass, neck circumference, exercise.

INTRODUCTION

Obesity is strongly associated with negative health outcomes, including metabolic syndrome (1), type-2 diabetes mellitus (2), cardiovascular disease (3) and cancer (4). Particularly, adverse changes of body composition with aging in adipose tissue have traditionally been associated with an increase of visceral adipose tissue (VAT) mass. Interestingly, neck adipose tissue (NAT), an ectopic deposit of the upper body adipose tissue accumulation that has remained at the shadow of VAT (5), has been independently related with increased cardiometabolic risk (CMR)(6; 7).

Upper body subcutaneous fat (UBSF) has been defined as a fat depot located in an anatomic compartment separate from abdominal subcutaneous fat (7). Interestingly, previous results have shown that a higher UBSF volume is strongly associated with adverse cardiometabolic risk factors, independently of BMI, neck circumference, or VAT mass (8), highlighting the importance of better understanding the role of this depot in the development of cardiometabolic diseases. Along this line, other authors have focused their efforts on determining how NAT ectopic accumulation might be modulating this non-understood cardio-metabolic role. Within the main findings, it has been shown that NAT is associated with higher CMR (6; 7), that NAT compartment volume (subcutaneous, intermuscular and perivertebral) increases with increasing adiposity, and that it follows a different pattern of accumulation according to sex (6), suggesting that neck adiposity also could be an indicator of poor long-term outcome (9). In addition, our unpublished results showed for the first time that NAT is associated with CMR and inflammatory profile, independently of VAT, and that neck circumference could be a useful subrogate tool of estimation of NAT and CMR, especially in young men. In this context, strategies that could help modulate or prevent NAT accumulation are of clinical interest.

Previous evidence has shown that different interventions of exercise are effective in the total and central adipose tissue reduction (10-12). In fact, effective elimination of excessive abdominal fat in obese people through weight reduction exercise programs has been associated with reductions in fasting plasma glucose, insulin levels, triglycerides, and HOMA score (12-14). Whether exercise may be able to reduce neck adipose tissue accumulation, and therefore help to counteract CMR through it, remains to be ascertained. Therefore, the aim of the present work was to examine the dose-response effect of a concurrent exercise intervention of 6 months on the neck adipose tissue and circumference of young healthy adults.

RESEARCH DESIGN AND METHODS

Study Design

The current study was a clinical trial, randomized (ClinicalTrials.gov, ID: NCT02365129), designed to compare the effects of vigorous and moderate exercise with control on neck adipose tissue and circumference among sedentary young healthy 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. All work was performed in accordance with the Declaration of Helsinki (2013 revision); all subjects gave their written informed consent, including their randomized group assignment. The participants' recruitment and intervention were conducted at the Sport and Health Joint University Institute (iMUDS) in Granada (Spain) during 2015-2016. Data analysis were performed between 2017-2019. In addition, nutritional counselling was not provided during the study period.

Study Participants

The participants were students from the University of Granada (Granada, Spain). All subjects had to: be 18-25 years old, have a body mass index (BMI) between 18.5-35 kg/m2, have a sedentary lifestyle (i.e., undertaking <20 min moderate-vigorous physical activity <3 days/week at baseline), be a non-smoker, take no medication, not be participating in a weight loss program, have a stable body weight over the last 3 months (changes <3 kg), have no cardiometabolic disease (e.g., hypertension or diabetes), and have no first-degree relative history of cancer.

Intervention Programs

Participants assigned to the moderate and vigorous exercise groups were instructed to participate for 6 months, whereas participants assigned to control group were instructed to not change their physical activity routine and diet.

However, the ACTIBATE group made the decision to offer the same physical training plan the year after completing the project.

The physical activity instructors between 2015-2016 realized the exercise intervention with a maximum of 16 persons. The participants had four different options of training schedule: 8:00 to 10:00 am, 16:00 to 18:00 pm, de 18:00 to 20:00 pm and 20:00 to 22:00. For special cases like holiday or absence to a session for an indefinite time, the instructions of aerobic and muscular strength training, together with a pulsometer and elastic band were gave.

The dose of exercise program was based in the physical activity recommendations for adults proposed by the World Health Organization (15), combining both aerobic and resistance training during 6 months. Briefly, the three characteristics components are summarized:

a) Volume: both the moderate-intensity and vigorous-intensity groups performed 150 minutes/week of aerobic exercise and ≈80 minutes/week of strength training. For aerobic exercise, the vigorous intensity group performed 75 minutes/week at moderate intensity (i.e. 60% of heart rate reserve (HRres)) and 75 minutes/week at vigorous intensity (i.e. 80% HRres), while the moderate-intensity group performed a total of 150 minutes/week of aerobic training at 60% HRres. The strength training was performed at 50% of 1 repetition maximum (RM) for the moderate-intensity group and at 70% RM for the vigorous-intensity group. An exercise heart rate monitor (RS800CX, Polar Electro Öy, Kempele, Finland) during the exercise sessions.

b) Intensity: The participants used a 60% HRres for the moderate-intensity group and 80% of HRres for the vigorous-intensity group, which increased gradually until these values were achieved. In addition, the intensity for the resistance training was 50% RM (maximum amount of weight one can lift in a single repetition) and 70% RM for the moderate-intensity and vigorous intensity group, respectively. The transition area of intensity was considered as all aerobic

activity between 65 to 75% HRres. In addition, the HR in the muscular strength exercise was not used to monitor the training intensity.

c) Frequency: The participants trained 3 or 4 days/week with the same weekly dose. In addition, the strength training was performed on 2 of these 3 or 4 days/week, and therefore 1 or 2 sessions per week consisted solely on aerobic exercise. Each participant had the freedom to select the kind of training.

For more details about characteristics components, see the methodology ACTIBATE study (16).

Study Outcomes

i) 18F-FDG-PET/CT

18F-fluordeoxyglucose (18F-FDG) positron emission tomography and of computed tomography scan (Siemens Biograph 16 PET/CT scanner) from the atlas vertebra to the mid chest region, were used to quantify neck adipose tissue (NAT) volume and distribution. All the subjects confirmed that they had met the next requirements: 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) having not consumed any alcoholic or stimulant beverages in the previous 6 h or taken any drugs that might affect the peripheral circulation in the last 24 h. The original aim of the ACTIBATE study (16) was to detect the volume and activity of brown adipose tissue (BAT). In consequence, the participants were submitted to a 60 min personalized cooling protocol prior to inject a bolus of 18F-FDG positron emission tomography with the objective to stimulate BAT metabolic activity (17). After 1 hour, with the participants in supine position and a thin pillow below their heads, a low dose CT (120 k) scan was performed for attenuation correction and anatomic localization. Immediately thereafter, one static acquisition of 2 PET bed positions (6 min each) was performed.

ii) Neck measurements quantification

For the quantification of NAT the CT component of the PET/CT was used, using the Beth Israel plugin for FIJI software http://sourceforge.net/projects/bifijiplugins/ by a single researcher (JMPG). Using a 3D-axial technique, several regions of interest (ROIs) at level of C5 were outlined to determine the NAT volume and the distribution of fat across the different NAT compartments. The NAT volumes in these ROIs were calculated by determining the number of pixels within the radiodensity range of -300 to -10 Hounsfield Units (HU). The NAT compartments were defined as:

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 vertebrae C5.

Besides, another ROI with pixels between 300 to -10 HU and 9 to 150 HU radiodensity ranges to total NAT and neck lean tissue (including skeletal muscle tissue, blood vessels, and some internal organs) (18) were considered, respectively.

Neck circumference (NC) was measured perpendicular to the longitudinal axis of the neck, using an inextensible metallic tape over the thyroid cartilage (19). The subjects were in an anatomical position, standing or sitting with the head in the Frankfort plane and the shoulders relaxed.

iii) Anthropometry and body composition

Weight and height were measured using a model 769 calibrated digital scale and a portable model 213 stadiometer, both from SECA (Hamburg, Germany), respectively. After, BMI was calculated as body weight (kg)/height squared (m2). Waist circumference was measured at the minimum perimeter, or when subjects showed abdominal obesity, in a horizontal plane above the umbilicus (19). Later, a dual-energy X-ray absorptiometry scanner (Discovery Wi, Hologic, Inc., Bedford, MA, USA) was used to determine lean mass, fat mass and visceral adipose tissue (VAT). We calculated lean mass index (LMI) as lean mass in kg divided by height2 in meters, and fat mass index (FMI) as fat mass in kg divided by height2 in meters. In addition, the analyses were conducted following the manufacturer's recommendations. The lean and fat mass and percentage of the trunk and appendicular regions were determinate (20).

Sample size

Assuming a power of >80%, error α of 0.05 and maximum loss at follow-up of 30%, 50 participants for each of the next study groups: control, moderate-intensity and vigorous-intensity were initially calculated in ACTIBATE project. After to pre-screening inclusion criteria, 194 participants were eligible for baseline assessment of which 145 were finally randomized in 54, 48 and 43 for control, moderate and vigorous groups, respectively. However, the participants included in the primary analysed were who did not have problems in the neck CT scan not and yes compliance the criterion of exercise intervention (explained in the statistical section).

Randomisation

Eligible trial participants were randomly assigned to control, moderate exercise, or vigorous groups for 6 months. The randomization was simple (unrestricted),

generated using xxxx statistical software, and was concealed until an eligible participant was ready for enrollment.

Blinding

The assessment staff was blinded about participant randomisation assignment with the aim to not interfere in the follow-up measurements. On the other hand, the participants were informed about the study hypotheses and the intervention group, although they were reminded frequently not to disclose their randomisation assignments.

Statistical analysis

The distribution of the variables was verified using the skewness, kurtosis values, visual check of histograms and Shapiro–Wilk test. All neck outcomes presented non-normal distribution (an exception of neck circumference). However, for a preliminary analysis the variables were used without being transformed. Hence, descriptive parameters were reported as median and interquartile ranges (between percentile 25 to 75). Due to the small sample size of each exercise group, results of women and men were pooled together.

One filter of exercise intervention was used. This included those participants who attended more than 70% of the total training sessions and adhered to the training intensity established for each group (i.e., considered as spending more than 50% of the time during aerobic exercise in moderate intensity, and less of 20% of the remaining time in the transition intensity area), or spending more than 40% of the time in aerobic exercise in vigorous intensity). Only those participants who complied with this criterion were included in the analyses.

For the analyses an ANOVA via bootstrapping (95% bias corrected and accelerated confidence intervals based on 1000 bootstrap samples) was performed adjusting for potential confounders: model 0 (unadjusted), model 1

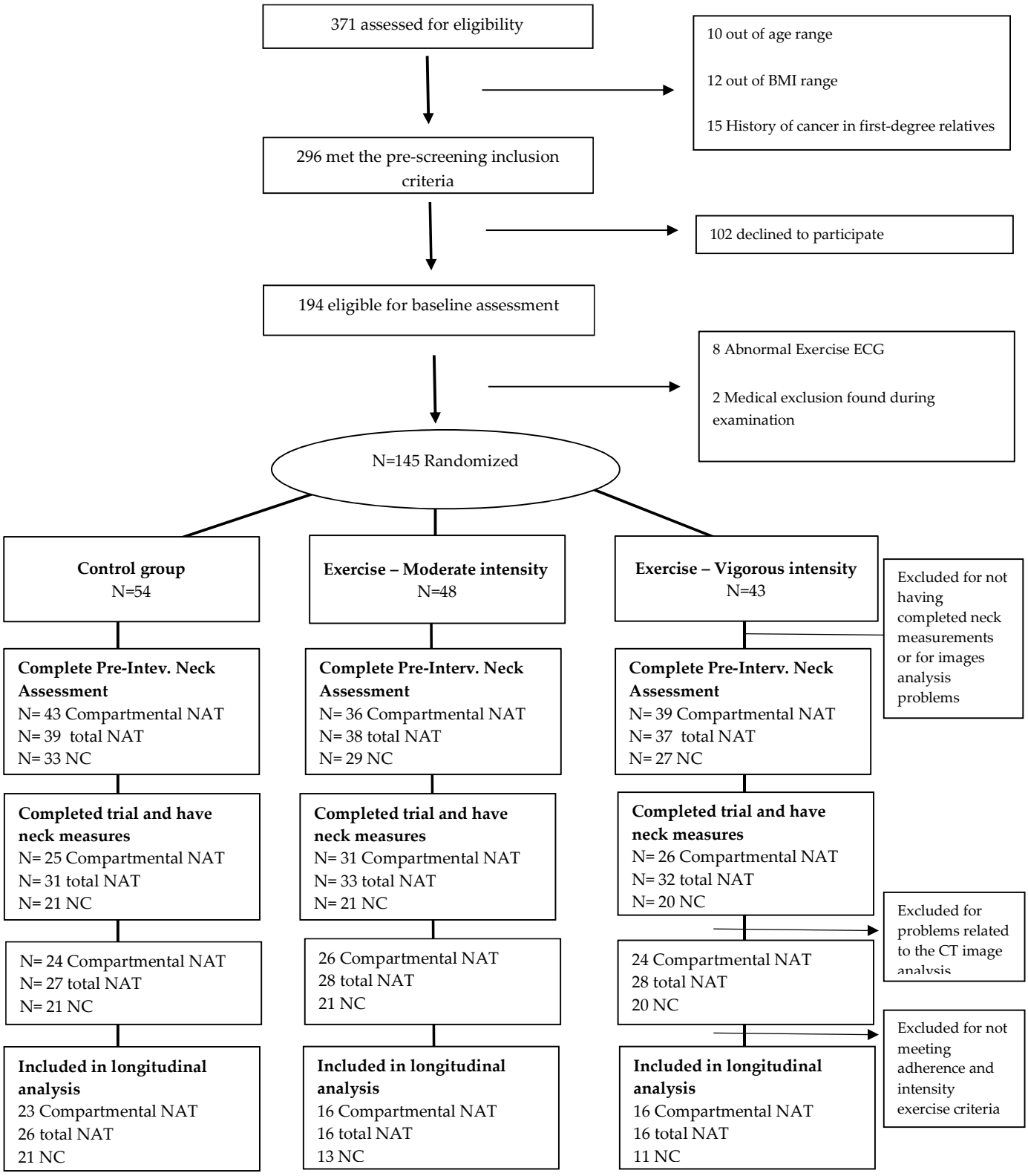
(adjusted for the baseline values) and model 2 (adjusted for the baseline values and sex). Mean differences and confidence intervals values are shown.

All-statistical analyses were performed using SPSS software version 21.0 (SPSS, Chicago, IL, USA). Significance was set at $P<0.05$.

RESULTS

Of the 194 subjects that were enrolled initially in the exercise intervention, 145 participants were randomized 54, 48 and 43 for control, moderate and vigorous groups, respectively. However, not all participants completed the 18F-FDG-PET/CT scanning to quantify their upper body adipose tissue. In addition, at the end to the intervention and after checking the CT scans and apply the exercise filter, the groups included in primary analysis were i) Control group: with 23 participants for compartimental NAT, 26 for total NAT and 21 for NC; ii) moderate group: 16 participants for compartimental NAT, 16 for total NAT and 13 for NC and iii) Vigorous group: 16 participants for compartimental NAT, 16 for total NAT and 11 for NC (see figure 1. Flow chart).

Figure 1. Flow chart ACTIBATE study



Flow-chart for subject enrolment. BMI: body mass index, ECG: electrocardiogram, NAT: neck adipose tissue, NC: neck circumference, PET/CT: positron emission tomography combined with computed tomography.

Table 1 shows the descriptive characteristics of the study participants.

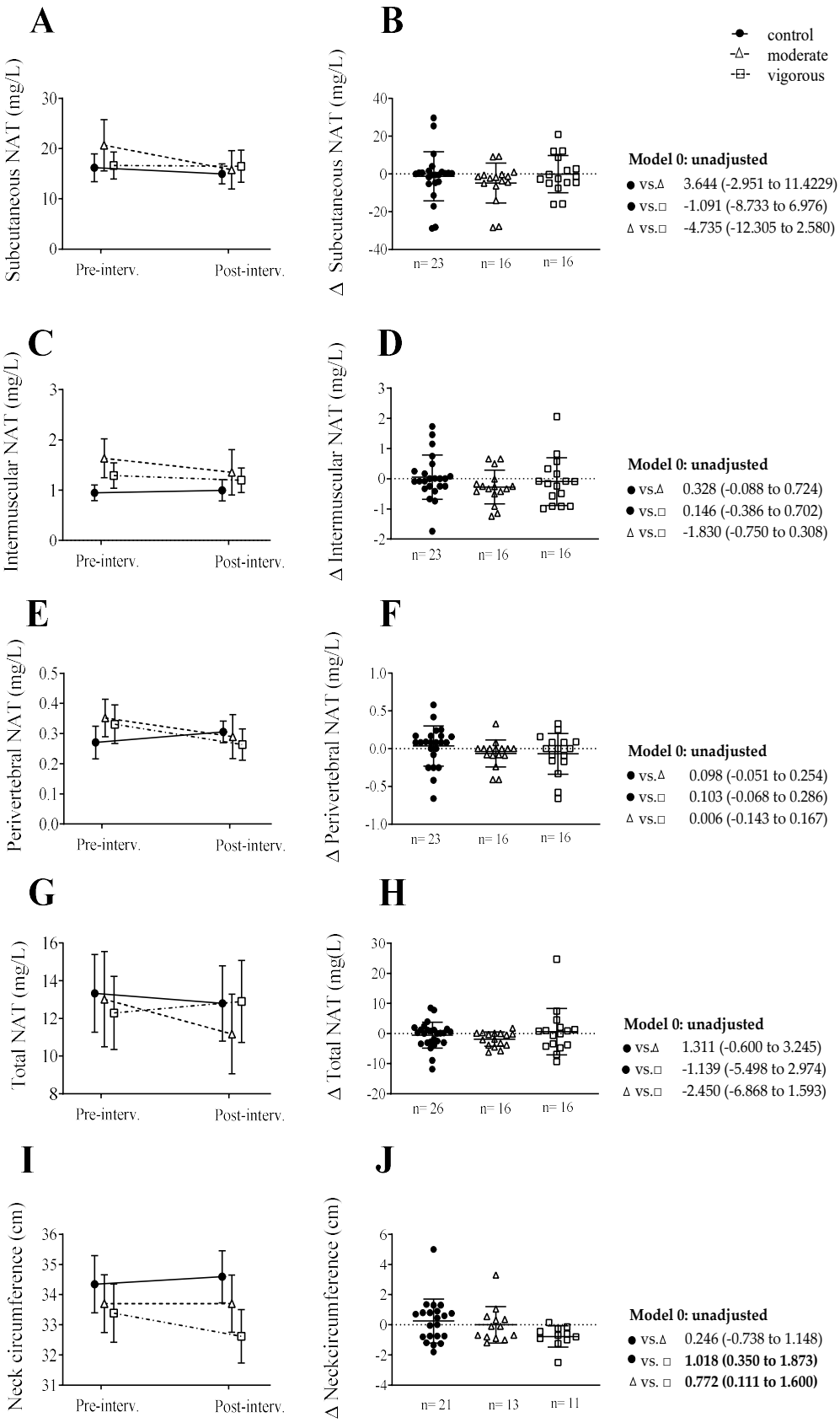
Table 1. Characteristics descriptive of the sample.

	Pre-intervention								Post-interventi	
	Control		Moderate		Vigorous		Control		Moderate	
Age (years)	22,1	(19,9- 24.0)								
Sex (% women)	60%		60%		72%		58%		72%	
Anthropometry and body composition										
Weight (kg)	66.9	(57.1-77.7)	57	69.0 (58.9-76.3)	20	67.7 (57.6-80.5)	23	68.9 (57.7-81.1)	38	70.2 (59.4-73.5)
Height (m)	1.67	(1.61-1.75)	57	1.69 (1.63-1.73)	20	1.66 (1.61-1.72)	23	1.68 (1.60-1.75)	38	1.69 (1.62-1.73)
BMI (kg/m^2)	23.2	(21.0 -26.6)	57	23.2 (20.7-27.3)	20	24.8 (22.63-27.84)	23	23.5 (21.7-26.7)	38	23.1 (20.7-26.9)
WC (cm)	77.7	(70.0-89.8)	56	80.0 (67.8-94.7)	20	78.4 (71.99-87.66)	22	81.4 (71.8-89.5)	38	76.2 (68.0-84.8)
Lean mass (kg)	38.8	(34.4-51.3)	57	40.2 (34.9-48.6)	20	37.1 (33.46-47.41)	23	40.5 (32.7-54.1)	38	40.9 (36.2-49.2)
Fat mass (kg)	22.1	(16.8-30.2)	57	23.0 (16.2-32.2)	20	24.3 (19.90-30.03)	23	21.3 (17.8-26.6)	38	19.6 (15.1-26.1)
Fat mass (%)	33.6	(29.4-40.2)	57	35.2 (28.8-43.4)	20	38.3 (35.03-41.32)	23	33.2 (28.3-38.9)	38	32.8 (26.2-37.8)
VAT mass (g)	303.6	(178.4-432.3)	57	297.6 (190.7-461.5)	20	314.4 (240.65-489.64)	23	311.5 (164.2-387.1)	38	228.2 (174.7-365.3
Neck measurements										
Subc. NAT (mL)	13.2	(6.7- 27.1)	23	12.4 (8.2-31.0)	16	17.8 (6.5-22.3)	16	12.71 (6.1-25.0)	23	11.2 (6.2
Interm. NAT (mL)	0.8	(0.3-1.3)	23	1.1 (0.6-2.2)	16	1.1 (0.3-2.2)	16	0.74 (0.25-1.2)	23	0.7 (0.3
Periv. NAT (mL)	0.3	(0.1-0.3)	23	0.3 (0.19-0.48)	16	0.3 (0.1-0.5)	16	0.25 (0.2-0.4)	23	0.3 (0.
Total NAT (mL)	9.5	(5.0-19.6)	26	8.6 (5.1-20.9)	16	12.0 (4.8-19.2)	16	9.78 (4.8-19.2)	26	7.4 (5.0
NC (cm)	32.8	(30.8-37.8)	33	33.1 (30.7-37.5)	14	34.0 (30.4-34.6)	11	34.2 (31.2-38.5)	21	33.0 (31.0

Median values (interquartile range between percentile 25 to 75) and sample sizes are provided, BMI: body mass index, Interm: i
NC: neck circumference, P: p-value, Periv: perivertebral, Subc: Subcutaneous, VAT: visceral adipose tissue, WC: waist circumfe
to compare the anthropometry, body composition and neck measurements variables of baseline across exercise groups. P-value

Figure 2, illustrates the significant effect that exercise intervention had only on neck circumference, but not in compartmental and total NAT (see Figure 2). The mean of change was significantly different between the control versus (vs.) vigorous group (mean change: 1.018 mL; confidence interval (CI): 0.350 to 1.873) and moderate vs. vigorous group (0.772 mL; CI: 0.111 to 1600) in the unadjusted model (model 0). These differences still remained (data not show) after to adjust by baseline (model 1) and baseline -sex (model 2). No significant associations were found between the rest of NAT measurements and exercise groups (see figure 1, Panels B, D, F, and H).

Figure 2. Changes in neck compartments (Subcutaneous, Intermuscular and Perivertebral), total NAT and neck circumference before and after the intervention across the different groups.



Only those participants who attended to more than 70% of total training sessions and adhered to the training intensity established for each group (i.e., considered as spending more than 50% of the time during aerobic exercise in moderate intensity, and less of 20% of the remaining time in the transition intensity area moderate), or spending more than 40% of the time in aerobic exercise in vigorous intensity vigorous) were included. Data on the left panels (A, C, E, G and I) are show as means ± standard error. Data on the right panels (B, D, F, H and J) show the change on the parameters of interest for each participant. An ANOVA via bootstrapping analyze (95% bias corrected and accelerated confidence intervals based on 1000 bootstrap samples) was performed. Mean differences and confidence intervals values are shown. NAT: neck adipose tissue, vs.: versus.

Sentitivity analyses

We repeated the analyses apply two less strict filter of exercise. The filters included in the analyses were:

i) Those participants who attended more than 70% of total training sessions and adhered to the training intensity established for each group (i.e., considered as spending more than 65% of the time during aerobic exercise in moderate intensity, or spending more than 40% of the time during aerobic exercise in vigorous intensity / more than 30% of this time in vigorous intensity and more than 30% of the remaining time in the transition intensity area vigorous) or

ii) Those participants who attended more than 70% of total training sessions.

When the participants attended more than 70% of sessions and adhered to the intensity established, we observed an effect of exercise on Δ total NAT and Δ neck circumference (see figure S1, Panels H and J, respectively). Differences of means between control and moderate groups were found in total NAT for the baseline (model 1) and baseline-sex adjusted model (model 2), (1.743 mL; CI: 0.0007 to 3.586 1.725 mL; CI: 0.011 to 3.406, respectively). Besides, for neck circumference, the unadjusted (model 0) showed a difference of means between control vs. moderate and control vs. vigorous groups (1.014 mL; CI: 0.278 to 1.842 and 0.905 mL; CI: 0.130 to 1.695, respectively). Similarly, values of mean neck circumference also were significantly different between control vs. vigorous and moderate vs. vigorous groups (1.141 mL; CI: 0.382 to 1.839 and 1.413 mL; CI: 0.530 to 2.483, respectively) after adjusted by baseline (model 1) and baseline-sex (model 2). No significant associations were found between the rest of NAT measurements and exercise groups (see 3, Panels B, D, and F).

Finally, we repeated all the analyses using a filter less rigorous (who attended to more than 70% of total training sessions). Differences of the mean between control and vigorous group, for the model unadjusted (model 0) of intermuscular

and perivertebral NATs (0.603 mL; CI: 0.036 to 1.329 and 0.149 mL; CI: 0.001 to 0.285, respectively), were observed (see figure S2, Panels D and F). In addition, values mean of neck circumference again were significantly different between control vs. vigorous and moderate vs. vigorous groups (see figure S2, Panel J) of the models unadjusted (model 0), baseline adjusted (model 1) and baseline and sex adjusted (model 2).

Sensitivity analyses are provided in Figure S1 and S2 of the Supporting Information.

DISCUSSION

Our preliminary results show, for the first time, the small effect of an exercise intervention on neck measurements in young healthy Spanish adults. Specifically, 6 months of moderate and vigorous exercise could generate a mean reduction of 1.01 and 0.77 centimeters of neck circumference, respectively. However, additional changes of NAT compartments were observed when the filter for session compliance was less restrictive. Consequently, these results give of entry point to future intervention in the management of upper body obesity. However, these findings must be taken with caution.

The increase of studies that incorporate neck circumference on the body composition adiposity indicators on exercise interventions could facilitate the understanding of the results. Along this line, Zanetti et al. (21) showed that only 12 weeks of resistance exercise in 15 participants with human immunodeficiency virus (HIV) and a higher risk of cardiometabolic disease consequence of its antiretroviral, decreased the neck circumference in 0.7 cm with respect to control group. Similarly, but with 16 weeks of resistance training in sarcopenic and non-sarcopenic obese elderly women, Oliveira Silva et al. (22) reported that only the non-sarcopenic group had decreased the neck circumference in 0.6 cm. Interestingly, similar to our intervention study, they did not do nutritional counselling. However, when the intervention utilized a dietary management the change in neck circumference can be more pronounced. Desplan et al. (23), show in 11 sedentary participants between the ages of 35 and 70 with metabolic syndrome and obstructive sleep apnea syndrome that only 4 weeks of an individualized exercise training program (1 session per day; 6 sessions per week during lasting 2 hours) significantly decreased neck circumference in 1.5 cm. Similarly, 8 weeks of regular physical activity (200 min/week) together with dietary re-education in sedentary overweight/obese women also has shown that a change of NC also is associated with a change of resting energy expenditure

(REE), suggesting that NC reduction might also reflect a reduction of REE (24). However, these results would be interpreted with prudence because they can represent a reduction of lean mass or other factors of REE. In addition, other studies also have shown that neck circumference did not change after 8 weeks of aerobic exercise in sedentary participants with overweight and moderate -severe obstructive sleep apnea syndrome (25), neither after 12 weeks of resistance training in 18 men adolescents and 33 women with obesity (26; 27).

Despite the previous results showing that neck circumference could be a subrogate measure of neck adipose tissue due its strong and moderate-to-strong associations with the NAT volumes in both men and women and similar correlations with CMR, in this study we did not find changes in NAT measurements. Nevertheless, changes between control versus moderate intensity on total NAT for age and sex, and between control versus vigorous over intermuscular and perivertebral NAT in the unadjusted model, were found when we used filters less strict of assistance and adherence intervention of exercise. The lack of previous evidence examining the influence of exercise on NAT preclude us from performing any comparison. Even though these changes are small, they could suggest the importance of the effect of intensity of exercise over lipolysis and mobilization of adipose tissue deposit in the neck and his relevant about CMR. To better understand the effect of the exercise on upper body fat mobilization, studies in vitro have shown that isolated visceral adipocytes (with high beta-adrenergic receptors) in non-obese women and men are 10-20 times more lipolytic sensitivity from both sexes than gluteal adipocytes (28). In addition, previous evidence shows that women have 10 to 15 times lower affinity in alpha 2-adrenoceptor (antilipolytic) of abdominal fat cells compared with gluteal cells (28; 29). In fact, in the abnormality context, previous experimental evidence has concluded upper body fat could contribute with >50% of FFA circulating and that women and men with central obesity delivery greater

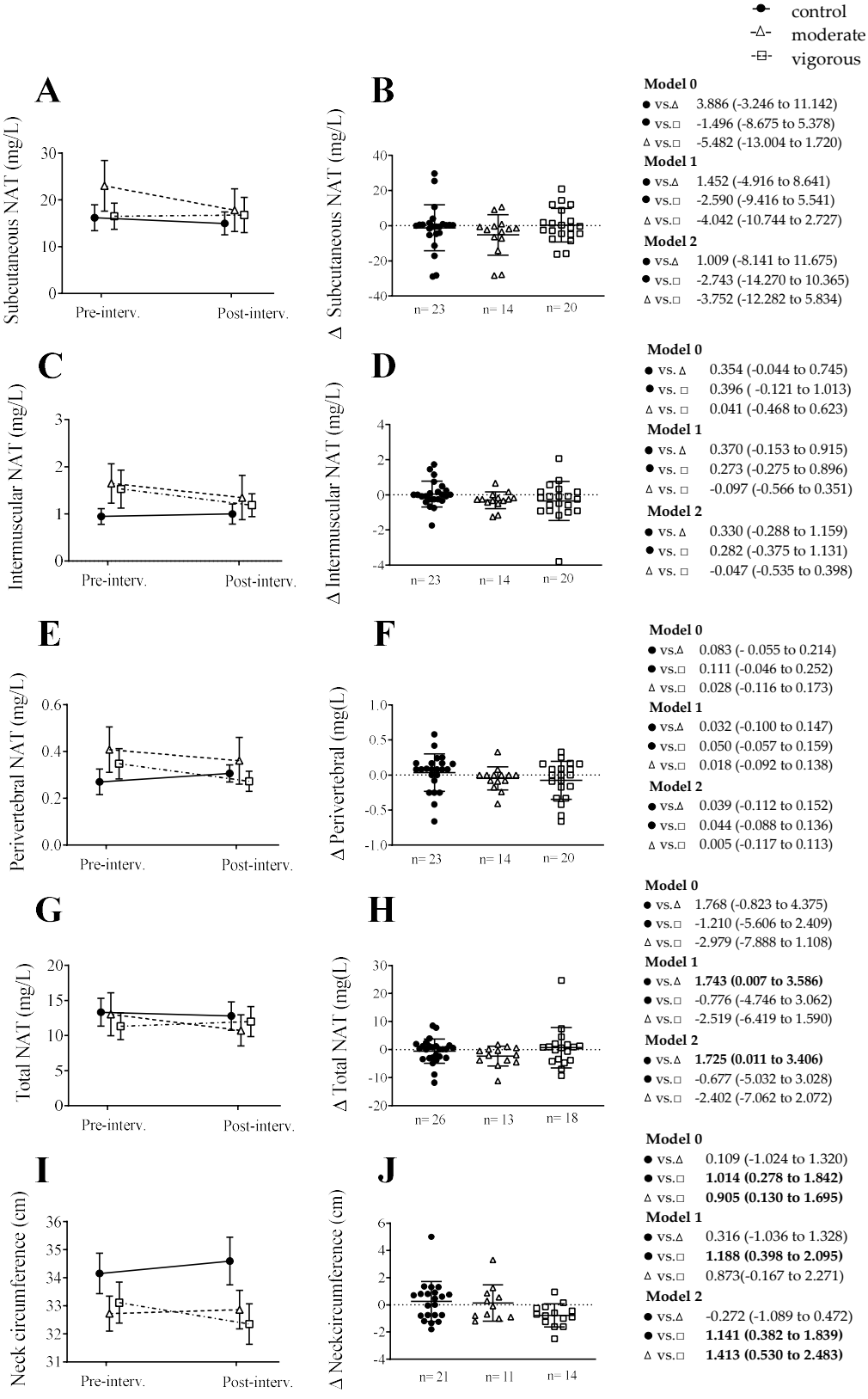
portion fat-free acids (FFA) from visceral adipose tissue to the liver than lean people, making this relationship more accentuated in women (30).

Despite that our preliminary findings are novel, they must be interpreted with caution. The results cannot be generalized to people with excess upper body fat due to the difficulties of accurately outlining the ROIs for distinguishing specific NAT compartments. In fact, since a thin pillow was placed below 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. In addition, the subjects underwent a personalized cold exposure prior to their PET/CT scan, which might have had a very small effect (cold exposure only induces a mean change of only ~3 HU)41 on the radiodensity readings, leading to a small number of voxels that should have been classified as NAT. On the other hand, the small number of participants in each group did not allow performance of the analyses separating women and men, which did not allow us to fully account for the potential effect of sex on the variables of interest. Future works should control the ingest of food and hydratation during exercise sessions, which have can have effect over lipolysis and examine the molecular signatures of the neck region to try to reveal the underlying mechanisms (e.g., lipid metabolism and regulation) by which the intensity of exercise has an effect over lipolysis of NAT.

In conclusion, our results show for the first time, that an exercise intervention of 6 months of moderate and vigorous intensity can slightly reduce neck circumference in young healthy adults. In addition, NAT measurements also could be decreased when less restrictive filters for session compliance are used. In consequence, these findings must be taken with caution.

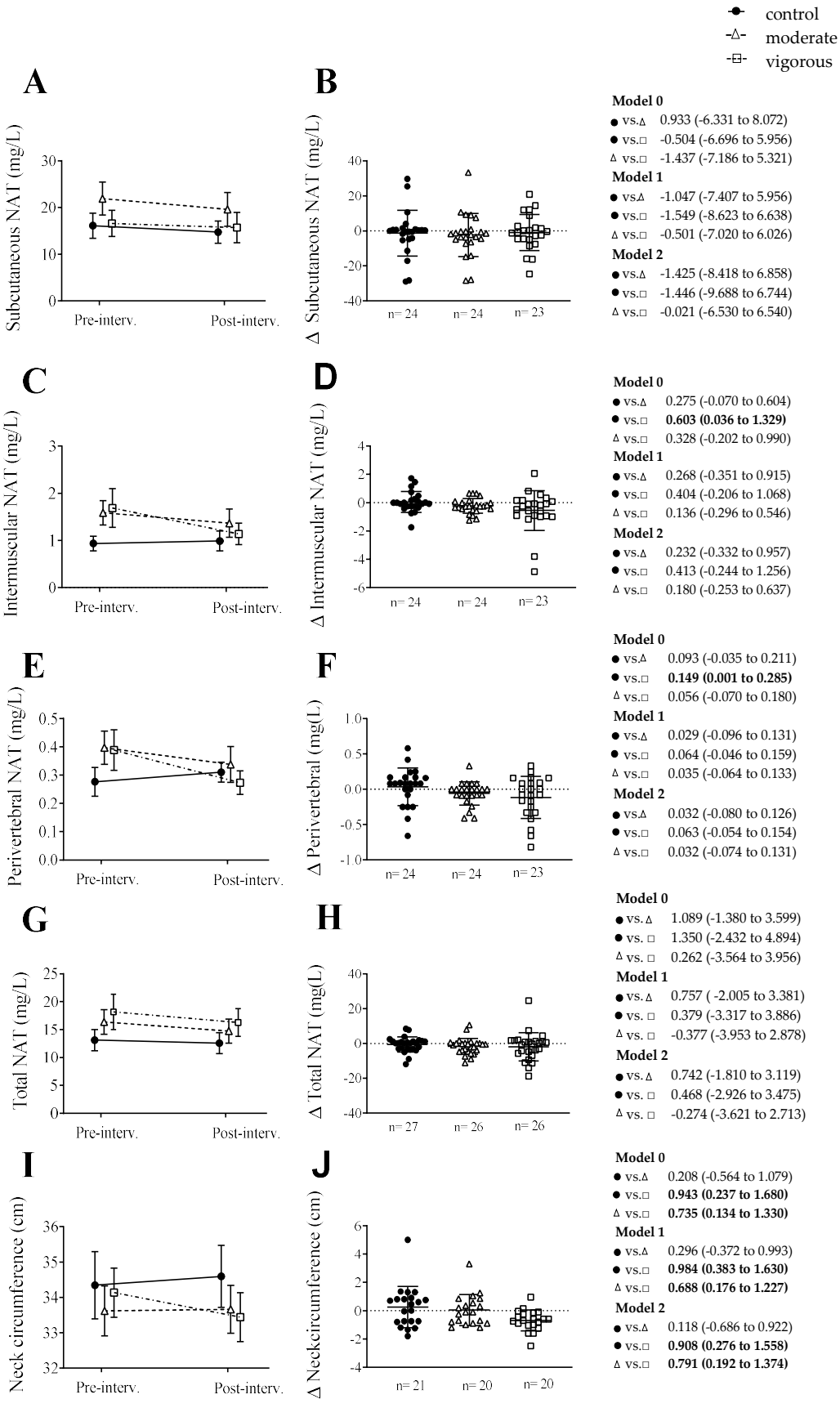
SUPPORTING INFORMATION

Figure S1. Changes in neck compartments (Subcutaneous, Intermuscular and Perivetebral), Total NAT and Neck circumference before and after the intervention across the different groups.



Only those participants who attended to more than 70% of total training sessions and adhered to the training intensity established for each group (i.e., considered as spending more than 65% of the time during aerobic exercise in moderate intensity, or spending more than 40% of the time during aerobic exercise in vigorous intensity / more than 30% of this time in vigorous intensity and more than 30% of the remaining time in the transition intensity area were included. Data on the left panels (A, C, E, G and I) are show as means ± standard error. Data on the right panels (B, D, F, H and J) show the change on the parameters of interest for each participant. An ANOVA via bootstrapping analyze (95% bias corrected and accelerated confidence intervals based on 1000 bootstrap samples) was performed adjusting for potential confounders: model 0 (unadjusted), model 1 (adjusted for the baseline values) and model 2 (adjusted for the baseline values and sex). Mean differences and confidence intervals values are shown. NAT: neck adipose tissue, vs.: versus.

Figure S2. Changes in neck compartments (Subcutaneous, Intermuscular and Perivetebral), Total NAT and Neck circumference before and after the intervention across the different groups.



Only those participants who attended to more than 70% of total training sessions were included. Data on the left panels (A, C, E, G and I) are show as means ± standard error. Data on the right panels (B, D, F, H and J) show the change on the parameters of interest for each participant. An ANOVA via bootstrapping analyze (95% bias corrected and accelerated confidence intervals based on 1000 bootstrap samples) was performed adjusting for potential confounders: model 0 (unadjusted), model 1 (adjusted for the baseline values) and model 2 (adjusted for the baseline values and sex). Mean differences and confidence intervals values are shown. NAT: neck adipose tissue, vs.: versus.

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06

GENERAL DISCUSSION

GENERAL DISCUSSION

This section provides a general discussion of the main results presented across the studies of this doctoral thesis, insights into relevant methodological considerations, and proposes future directions for the field.

In the thesis, our results show that the correlation of neck circumference with indicators of body composition measured by DXA is lower than that which was observed for other classic anthropometric indicators such as BMI and WC, but suggests the utility of neck circumference as IMAT accumulation in premenopausal women. Besides, we found in sedentary young people, that a higher BMI was directly associated with higher NAT depot, together with highlighting the direct association between intermuscular and total NAT depot with CMR and pro-inflammatory status. In addition, our findings support the utility of neck circumference as a measure of estimation of NAT and CMR, especially in men.

Additionally, we conclude for first time, that higher sedentary time and lower intensity of physical activity are associated with higher intermuscular and total NAT depot in men, remarking the importance of the sexual dimorphism above characteristics and mechanisms of adipocyte of the neck. In this context, our preliminary results over the effect of a concurrent exercise intervention on NAT also show the importance of 6 months moderate or vigorous exercise therapy in a slight reduction of neck circumference of young adults. However, these findings must be interpreted with precaution.

The results of study 1 and study 3 are in line with the results recently showed by Castro-Pinero et al (1). They showed an association of neck circumference with cardiovascular disease risk factors and insulin resistance, and include a number of anthropometric and body composition indices derived from skin-fold thickness in Spanish children and adolescents. However, they found weaker

associations between neck circumference and fat mass index (FMI) in girls ($r=0.494$, $P<0.001$) and in boys ($r=0.474$, $P<0.001$) most likely due to the fact that they estimated fat mass from skin-fold thickness. In addition, the authors stablished a neck circumference of 33.5 and 37.1 cm in girls and boys respectively as the cut-off to classify youths of 18 years as overweight/obese, which concur with the mean values of our study participants. On the other hand, despite that neck circumference has been defined as a practical indicator for identifying adult people with overweight and obesity (2-5), previous studies have concluded that it would not be better than BMI and WC (6; 7). Similar results were found in study 1, however our findings showed that neck circumference was associated with FMI, VAT and lean mass index LMI, independently of BMI, highlighting the importance of using this measure in combination with BMI for future studies, especially with women.

The lack of previous evidence does not allow us to generate a comparative analysis of the findings of study 2. We showed, for first time, that a high neck circumference is associated with thigh IMAT volume and VAT in overweight and obese premenopausal Caucasian women. These results are totally novel and remark the utility of this anthropometry measure (simple, low cost and feasible), as an alternative indicator of adipose tissue content in thigh skeletal muscle. However, they suggest the necessity of future research about this topic.

More recently, NAT, which has been defined as a complex subcutaneous fat depot of the upper region of the body, remained somehow in the shadow of other more investigated depots (ex. VAT) (8). In line with previous studies (9-11), we show that NAT seems to be an ectopic fat depot that expands with increasing adiposity, and is related to a higher CMR and, for the first time, a pro inflammatory status independently of BMI, body fat percentage and VAT in sedentary young people. In addition, our results suggest that total NAT volume might have a similar predictive value of the CMR and inflammatory status than

VAT mass, and support the utility of neck circumference as a clinical screening tool in the estimation of CMR, especially in men. Interestingly, the results did not change after applying False Discovery Rate (FDR) correction.

The importance of study 4 would have a direct relationship with the relevance of increased knowledge and understanding about the environment of sedentary behavior and physical activity intensities in the neck fat depots. The lack of literature between sedentary time, physical activity and NAT, makes the comparison across studies difficult (12-14). Nevertheless, the results of previous studies (12-14) that highlight the importance of sexual dimorphism over lipolysis and body fat distribution of upper body subcutaneous fat, could help explain part of our findings. In this context, our results suggest that the adipocyte of the neck could have similar characteristics of size, HSL and B-adrenergic receptor than VAT (15). This could remark the importance of incorporate analyses of biopsies of NAT depots in future studies. Additionally, the positive association between several physical activity categories with the compartmental and total NAT volumes in women highlight the need to replicate this methodology in different groups of women. Besides, considering that interventions designed to change lifestyle behaviors with the aim of meeting international physical activity recommendations, future studies about this topic could be very important in future therapy and public decision to combat obesity and comorbidities related to accumulation of neck fat. However, the results should be interpreted with caution given that a small number of males were included and that all associations became non-significant after adjusting for multiplicity by FDR.

Finally, novel results about NAT, neck circumference and exercise are shown. Interestingly, in study 5 we show that the concurrent exercise of intensity moderate and vigorous could slightly decrease neck circumference in a mean of 1 cm after 6 months of intervention in young healthy Spanish adults. Due to the preliminary character of our results these findings must be considered with

caution. Indeed, these significant associations were observed when the more exigent criteria about compliance of intensity and adherence to the program was used. This included those participants who attended more than 70% of the total training sessions and adhered to the training intensity established for each group (i.e., considered as spending more than 50% of the time during aerobic exercise in moderate intensity, and less of 20% of the remaining time in the transition intensity area), or spending more than 40% of the time in aerobic exercise in vigorous intensity. However, changes over NAT measurements were observed, when i) the participants attended more than 70% of sessions and adhered to the intensity established (i.e., considered as spending more than 65% of the time during aerobic exercise in moderate intensity, or spending more than 40% of the time during aerobic exercise in vigorous intensity / more than 30% of this time in vigorous intensity and more than 30% of the remaining time in the transition intensity area vigorous) or ii) when they attended more than 70% of total training sessions. In consequence, new statistical analyses (without outliers and less robust) must be considered.

In conclusion, NAT distribution and neck circumference and their relation with CMR factors and lifestyle behavior in young sedentary people, actually are on topic thematic of study. However, if these results can be found in children, pregnant women and older people, are not yet known. Therefore, despite little studies in healthy populations, our results remark the importance of increasing the understanding of this complex fat depot at the shadow of other deposits extendedly investigated, together while highlighting the utility of neck circumference and NAT in future clinic interventions.

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07

FUTURE PERSPECTIVES

FUTURE PERSPECTIVES

The integrative future perspectives in this area of study are describe continuation:

- To increase studies in this topic together with examining other populations of interest (children, adolescents and elderly people).
- For future studies it is important to reduce limitations of NAT estimation. For example: not to use a pillow during TC scan.
- To increase sample size to improve statistical power.
- To determine the cut off of neck circumference for total NAT excess.
- The absence of knowledge about characteristics of white adipose tissue in the neck depot does not allow a clear understanding of the mechanism that explains own finding. For this reason, it is necessary to increase efforts to realize integrative studies that include molecular biology (biopsies').
- The nutritional aspects (ex. hydration or carbohydrate ingest) that can interfere in the fat oxidation and mobilization during the pre and post-exercise must be managed in a future intervention.

08

OVERALL LIMITACIONS AND STRENGHTS OF THIS DOCTORAL THESIS

OVERALL LIMITATIONS AND STRENGHTS OF THIS DOCTORAL THESIS

The limitations and strengths of the studies included in the present PhD Thesis are presented in Table 1.

Table 1. Limitations and strengths of the studies included in the present PhD Thesis

Study	Limitations	Stren
Cross-sectional ACTIBATE studies.	• This study applied a cross-sectional design, and therefore, drawing causal associations is not possible. • Sample size: - Differences between women and men (greater predominance of women). - Little sample size that not allowed determinate of cut-off of neck circumference of obesity and CMR screening. • Neck adipose tissue: - Volumetric quantification in the practical: might not be a viable option given the high cost, technical difficulties and radiation exposure of PET/CT imaging. - Results may not be generalizable for people who have an excess upper body fat (difficulties to accurately outline the ROIs for distinguishing specific NAT compartments).	• Use of D composition a • Population yo analyses of ne • We are the relationship b - Neck adip proinflamma - Neck adip sedentary physical acti - Neck adipo after exercise

- The personalized cold exposure prior to the PET/CT scan, might affected the radiodensity of the NAT, such that voxels initially classified as adipose tissue (i.e., within -10 to -300 HU). However, this is highly unlikely since cold exposure induces a mean change of only ~3 HU.
- Due at use of one pillow below their participant's heads (slightly inclined), we only could draw the compartmental ROIs in the posterior part of the neck in order to estimate NAT volume at the same height (C5 vertebrae).
- Unknowledge of finding as adipocyte characteristic (type, size and receptors), molecular signatures and mechanisms of action (ex. lipolysis) specific situations of the neck region.

- Potential confounders in the physical activity analyse (e.g., the type of PA or the pattern of movement performed [which cannot be distinguished by the accelerometer used]), might have partially affected the results.

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adipose tissue
- To analyse
behaviour an
with acceleron

Study	Limitations	Str
Cross-sectional pre-menopausal Women.	• This study applied a cross-sectional design, and therefore, drawing causal associations is not possible. • Our findings can only be generalized to Caucasian overweight/obese premenopausal women.	• To underscore techniques us assess IMAT with DXA m and regional b
Longitudinal study.	• Sample size: - Differences between women and men (greater predominance of women). - Differences between exercise groups after use different filter. • We do not control the nutritional ingest of macronutrients as carbohydrates (that can interferences over fat mass oxidation) pre and post exercise session.	• To design of s controlled tria • We are the fir findings abou concurrent ex on neck adipo adults.

09

OVERALL CONCLUSIONS

OVERALL CONCLUSIONS

This section offers the main conclusions of this doctoral thesis according to each study presented:

Conclusion 1 (study 1). Neck circumference is associated with anthropometric indicators (BMI, WC, TMI, W/Hip and Waist/height) and indicators of body composition measured by DXA (FMI, VAT, and LMI). Our results indicate that neck circumference is not a better predictor of total and central body fat than other classic anthropometric markers as BMI or WC in young healthy adults. Future studies should explore its usefulness as a measure to use in combination with BMI, especially in women.

Conclusion 2 (study 2). Neck circumference is associated with a higher amount of ectopic fat deposition in the thigh skeletal muscle, underscoring the relevance of NC as an indicator of adipose tissue content in thigh skeletal muscle. Neck circumference should be used as an alternative anthropometric measure to predict thigh IMAT (when BMI or WC are invalidated) in overweight and obese premenopausal women due to its simplicity, feasibility and low cost.

Conclusion 3 (study 3). Compartmental and total NAT volumes expands with increasing of adiposity, and associated with higher CMR and a more pro-inflammatory state in young healthy adults. More studies are necessary to understand the action mechanisms of NAT accumulation over cardiometabolic disease and inflammation state.

Conclusion 4 (study 4). The time spent in sedentary behaviour and PA of different intensity is associated with the compartmental and total NAT volumes in a sex-dependent manner. Interestingly, the men were who spent less time in sedentary behaviour and more in PA and had smaller compartmental and total NAT volumes. Future studies that examine how genetic and hormonal factors,

and other lifestyle behaviors, are related to specific sex patterns of NAT accumulation, are necessary.

Conclusion 5 (study 5). A 6-month exercise intervention of moderate and vigorous-intensity could reduce slightly the neck circumference in young healthy adults. However, due to the preliminary character of the results, these must be interpreted with caution.

10

ANEXES

Curriculum Vitae
Acknowledgements/
Agradecimientos

CURRICULUM VITAE

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- 2012-2020 (and currently). Academic Professor. Department of Nutrition, Faculty of Medicine, University of Chile, Santiago, Chile.

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SCHOOLAR SHIPS AND AWARDS

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  - 2016. **Carolina Foundation, PhD in Spain (4 years).**
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LENGUAGES, SOFWARES AND EQUIPAMENT HABILITATION

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  - 2016. English courses, University of Chile, Santiago of Chile.

- **Software:**

  Software: EVALFINUT. Evaluator of nutritional analyses.

  Level: Advanced

  Software: Temperatus. Body temperature assessor.

  Level: Intermediate

  Software: SPSS. Statistical Program Analyse.

  Level: Advanced

  Software: STATA. Statistical Program Analyse.

  Level: Intermediate

  Graph Prism. Analysis and graphing solution purpose-built for scientific research.

  Level: Advanced

- **EQUIPAMENT:**

  DXA, HOLOGIC Wii: Dual-energy X-ray absorptiometry for body composition evaluation.

  Level: Advanced

RESEARCH PROJECTS PARTICIPATION

- **PROJECT:** ***"Activating Brown Adipose Tissue through Exercise (ACTIBATE)"***
  - Occupation: Nutritionist, PhD Student
  - Lean Research: PhD Jonatan Ruiz Ruiz. PROFITH "PROmoting FITness and Health through physical activity" research group.

Department of Physical and Sports Education, Sport and Health University Research Institute (iMUDS), Faculty of Sports Science, University of Granada, Spain.

- Date: 2016-2020.

- **PROJECT:** ***"Preschool Fit-healthy and Smart: PREFIT-Chile Study Linking PhysicalFitness to non-invasive health-related markers and Executive Function".***

  - Occupation: Nutritionist PhD student
  - Lean Research: PhD Johana Soto, Department of Physical activity, Faculty of Sciences of physical activity and sport. University of Playa Ancha. Avenida Playa 850, Edificio Punta-Ángel, Valparaíso, Chile.
  - Date: 2018-2019.

- **PROJECT:** ***"Axial musculature control among climbers: sport performance determinant?".***
  - Occupation: Nutritionist. Body composition estimation.
  - Lean Research: Ignacio Solar Altamirano, High Performance Center. Pedro de Valdivia 4801, Ñuñoa, Chile.
  - Date: 2018-2019.

- **PROJECT:** ***"Growth and Obesity Cohort Study".***
  - Occupation: Master Student, Nutritionist.
  - Lean Research: PhD Camila Corvalán, Institute of Nutrition and Food Technology (INTA), University of Chile, Santiago, Chile.
  - Date: 2013-2016.

PhD TRAINING COURSES

- ***"Strategies to modify the writing, publication and communication of scientific articles"***. University of Granada. Granada, Spain.
  - Supervisor: Francisco Ortega and Jonatan Ruiz.
  - Duration: 20 classroom hours.
  - Year: 2020.

- ***"Systematic Revision Course"***. University of Granada. Granada, Spain.
  - Supervisor: Javier Sanz Valero de la Universidad Miguel Hernández y Carmina Wanden-Berghe de la Universidad CEU Cardenal Herrera.
  - Duration: 20 classroom hours.
  - Year: 2018

- ***"Investigation, innovation, intellectual propriety and knowledge transference"***. University of Granada. Granada, Spain.
  - Supervisor: José Antonio Morales Molina.
  - Duration: 20 classroom hours.
  - Year: 2018.
- ***"Analysis statistic of aleatory essays clinic".*** IMDS, University of Granada. Granada, Spain.
  - Supervisor: Jonatan Ruiz Ruiz.
  - Duration: 15 classroom hours.
  - Year: 2018.
- **"Statistical techniques in nutrition and health"**. University of Granada. Granada, Spain.
  - Supervisor: Francisco M. Ocaña Peinado y Fátima Olea Serrano.
  - Duration: 15 classroom hours.

  - Year: 2018.

- ***"Strategic and appreciation of critical nutrients in the diet"***. University of Granada. Granada, Spain.
  - Supervisor: Ángel Gil.
  - Duration: 6 classroom hours.
  - Year: 2017.

- ***"Missing Data".*** University of Granada. Granada, Spain.
  - Supervisor: María del Carmen Ruiz Ruiz.
  - Duration: 20 classroom hours.
  - Year: 2017.

- ***"Advances in Nutrition. IV Course".*** University of Granada. Granada, Spain.
  - Supervisor: Ángel Gil.
  - Duration: 12 classroom hours.
  - Year: 2017.
- ***"IV Course of summer FINUT: Healthy habits, strategic, investigation and perspectives".*** University of Granada. Granada, Spain.
  - Supervisor: Ángel Gil.
  - Duration: 20 classroom hours.
  - Year: 2017.

- ***"International course of sport nutrition. IINSS-IINCD (online modality)."*** International Institute of Nutrition and Sport Science.
  - Supervisor: Juan Mielgo-Ayuso and Aritz Urdampilleta.
  - Duration: 75 classroom hours.
  - Year: 2016.

- ***"International Anthropometry certification ISAK 2".*** University of Desarrollo, Santiago, Chile.
    - Supervisor: María Mercedes Dumont Ferro
    - Duration: 60 classroom hours.
    - Year: 2015.

- ***"Docent habilitation: Skill of teamwork".*** Faculty of Medicine, University of Chile, Santiago, Chile
    - Supervisor: Department of education in health sciences. Faculty of Medicine, University of Chile.
    - Duration: 27 classroom hours.
    - Year: 2015.

- ***"Selection multiple questions, Dr. Pedro Herskovic L".*** Faculty of Medicine, University of Chile, Santiago, Chile
    - Supervisor: Department of education in health sciences. Faculty of Medicine, University of Chile.
    - Duration: 16 classroom hours.
    - Year: 2014.

- ***"Instructor SVB/BLS Course. American Diabetes Association"***. Las Condes Clinic, Santiago, Chile.
    - Supervisor: Ignacio Solar Altamirano.
    - Duration: 20 classroom hours.
    - Year: 2013.

- ***"International Anthropometry certification ISAK 1"***. Santo Tomas University, Viña el Mar, Santiago, Chile.
    - Responsable: Miguel Salas Ávila.

- Duration: 27 classroom hours.
- Year: 2012.

ARTICLES IN JCR JOURNALS

- **Published**
  - **Arias Tellez MJ,** Silva AM, Ruiz JR, et al. Neck circumference is associated with adipose tissue content in thigh skeletal muscle in overweight and obese premenopausal women. Sci Rep. 2020; 10(1):8324.

  - **Arias Téllez MJ,** Acosta FM, Sanchez-Delgado G, Martinez-Tellez B, Muñoz-Hernández V, Martinez-Avila WD, Henriksson P, Ruiz JR. Association of Neck Circumference with Anthropometric Indicators and Body Composition Measured by DXA in Young Spanish Adults. Nutrients. 2020 Feb 18;12(2).

  - **Arias Téllez MJ**, Carrasco F, España Romero V, Inostroza J, Bustamante A, Solar Altamirano I. A comparison of body composition assessment methods in climbers: Which is better? PLoS One. 2019 Nov 20;14(11):e0224291. doi: 10.1371/journal.pone.0224291. eCollection 2019. PubMed PMID: 31747391.

  - **Arias Téllez MJ**, Carrasco Navarro GN, Plaza Díaz J. [Carbohydrate and lipid consumption before a training session changed the fat mass in health-physically active adults: a controlled and randomized clinical trial]. Nutr Hosp. 2018 Aug 2;35(4):936-941. doi: 10.20960/nh.1774. Spanish. PubMed PMID: 30070885.

- **Arias Téllez MJ**, Martinez-Tellez B, Soto J, Sánchez-Delgado G. [Validity of neck circumference as a marker of adiposity in children and adolescents, and in adults: a systematic review]. Nutr Hosp. 2018 Apr 5;35(3):707-721. doi: 10.20960/nh.1582. Review. Spanish. PubMed PMID: 29974783.

- **Arias Téllez MJ**, Soto-Sánchez JP, Weisstaub SG. Physical fitness, cardiometabolic risk and heart rate recovery in Chilean children. Nutr Hosp. 2018 Jan 10;35(1):44-49. doi: 10.20960/nh.1323. PubMed PMID: 29565148.

- Latin American Society for Pediatric Research (LASPR) Selected Abstracts From the L Annual Meeting. Pediatr Res 73, 378–380 (2013). https://doi.org/10.1038/pr.2012.181

- Submitted
  - **Arias Téllez MJ**, Acosta FM, García-Rivero Yolanda, Pascual-Gamarra JM, Merchán Ramírez E, Martínez-Téllez B, Silva MA, Almanza López J, Llamas-Elvira JM, Ruiz JR. Neck adipose tissue accumulation is associated with higher overall and central adiposity, a higher cardiometabolic risk, and a pro-inflammatory profile in young adults. Submitted to Journal Obesity (Journal Impact Factor: 4.514, Q1 Endocrinology & Metabolism and Nutrition and Dietetics).

  - **Arias Téllez MJ**, Acosta FM, Pascual-Gamarra JM, García-Rivero Yolanda, Merchán Ramírez E, Martínez-Téllez B, Silva MA, Almanza López J, Llamas-Elvira JM, Ruiz JR. Objectively measured sedentary time and physical activity are associated with neck adipose tissue.

Submitted to Obesity Journal (Journal Impact Factor: 3.969, Q1 Nutrition&Dietetics).

BOOK CHAPTERS

- Title: Dairy products ingest, Physical activity and your synergic effect about bone and muscle healthy. Book: Dairy products: Nutrition and health. Publication date: In process.

- Title: Chapter 15 - Use of Probiotics in Inflammatory Bowel Disease. January 2019
  DOI: 10.1016/B978-0-12-815249-2.00015-4. Book: Microbiome and Metabolome in Diagnosis, Therapy, and other Strategic Applications.

VISITING RESEARCHER

- 2019. January- February. Exercise and Health Laboratory, CIPER, Faculdade Motricidade Humana, Universidade de Lisboa, Estrada da Costa, 1495-688 Cruz Quebrada, Portugal. Advisor: PhD. Analisa Monica Silva.

- 2019. April- Jun. Department of Physical Education, Federal University of Paraíba, Brazil. Avenida Rio Grande do Sul, 748, Apt. 202, Edificio Acuarela, João Pessoa-PB, Brazil. Advisor: PhD. Clarice Martins.

CONGRESES PRESENTATIONS

- **Activity: "European Congress of Obesity"**
  - Role: poster communication: "Neck adipose tissue accumulation is associated with higher overall and central adiposity, cardiometabolic risk, and pro-inflammatory profile in young adults".

- Date: Sept, 2020.
- Place: Dublin, Irlanda.

- **Activity: "FINUT 2020 Conference"**
  - Role: poster communication: "Association of neck circumference with anthropometric indicators and body composition measured by DXA in young Spanish adults".
  - Date: Oct, 2020.
  - Place: Mexico City, Mexico.

- **Activity: "Workshop Acelerometry".** PROFITH "PROmoting FITness and Health through physical activity" research group. Department of Physical and Sports Education, Sport and Health University Research Institute (iMUDS), Faculty of Sports Science, University of Granada, Spain.
  - Role: poster communication: "Objectively measured sedentary time and physical activity are associated with neck adipose tissue volume in young sedentary adults".
  - Date: November, 2019.
  - Place: Granada, Spain.

- **Activity: "64◦ Congress of Chilean Medicine of sport"**
  - Role: poster communication: "A comparison of body composition assessment methods in climbers: Which is better?".
  - Date: August, 2019.
  - Place: Santiago of Chile.

- **Activity: "64◦ Congress of Chilean Medicine of sport"**
  - Role: poster communication: "lean mass and forearm strength in climbers. Are determinates of the performance? ".

- Date: August, 2019.
- Place: Santiago of Chile.

- **Activity: "International Symposium Role of Brown Adipose Tissue in Human Health".** PROFITH "PROmoting FITness and Health through physical activity" research group. Department of Physical and Sports Education, Sport and Health University Research Institute (iMUDS), Faculty of Sports Science, University of Granada, Spain.
  - Role: Scientific Committee.
  - Date: Nov, 2018.
  - Place: Granada, Spain.

- **Activity: "WORLD CONFERENCE ON KINANTHROPOMETRY - CHILE 2018"**
  - Role: poster communication: "Association of neck circumference with markers of body composition measured by DXA in young adults".
  - Date: Jun-Jul, 2018.
  - Place: Santiago of Chile.

- **Activity: "European Congress of Obesity"**
- Role: poster communication: "Association of brown adipose tissue, skeletal muscle glucose uptake and supraclavicular skin temperature, with cold-induced thermogenesis and nutrient oxidation rates".
  - Date: May, 2018.
  - Place: Vienna, Austria

- **Activity: "3◦ International Symposium of Exercise sciences and Physical Activity."**

- Role: poster communication: "Association of objectively measured sedentary time and physical activity with neck circumference in young adults".
- Date: April, 2018.
- Place: Santiago of Chile.

- **Activity: "Training Researchers Day: promoting interdisciplinary (JIFFI)"**
  - Role: poster communication: "Validity of neck circumference as a marker of adiposity in children and adolescents, and in adults: a systematic review".
  - Date: May, 2017.
  - Place: Granada, Spain.

- **Activity: "Latinoamerican Pediatric association of Investigation (SLAIP)"**
  - Role: poster communication: "Association between fitness and heart rate recovery in Chilean school".
  - Date: Oct, 2015.
  - Place: Cochabamba, Bolivia.

- **Activity: "60◦ Congress of Chilean Medicine of sport"**
  - Role: oral communication: Protein ingest in post exercise condition.
  - Date: August, 2015.
  - Place: Santiago of Chile.

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invitaron a compartir momentos importantes en sus vidas, viajes, me acogieron en sus casas y me ofrecieron ayuda en momentos difíciles: Jeff Oescher, Manolo Ortiz, Dani López, Elena, Hosam, Gracias de corazón a cada uno de ustedes. Ya saben que en Chile y Brasil, tendrán un lugar donde llegar cuando decidan ir☺.

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- **Clarice.** Amiga, você es um exemplo a seguir. Nunca troque sua forma do ser. Obrigada você y sua família por todo ló que vocês fizeram por eu em esse tempo.
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DOCTORAL PROGRAMME IN BIOMEDICINE

Neck adipose tissue and circumference as predictors of cardiometabolic risk in sedentary adults

María José Arias Téllez