International Doctoral Thesis / Tesis Doctoral Internacional

EFFECTS OF INTERVAL AEROBIC TRAINING COMBINED WITH STRENGTH EXERCISE ON CARDIOMETABOLIC MARKERS. TRANSFERRING FROM ANIMAL TESTING TO CLIMACTERIC WOMEN

EFECTOS DEL EJERCICIO AERÓBICO INTERVÁLICO COMBINADO CON FUERZA SOBRE MARCADORES CARDIOMETABÓLICOS. TRANSFERENCIA DE UN MODELO EXPERIMENTAL ANIMAL A MUJERES EN ETAPA CLIMATÉRICA

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IRENE COLL RISCO

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A las buenas personas

EFFECTS OF INTERVAL AEROBIC TRAINING COMBINED WITH STRENGTH EXERCISE ON CARDIOMETABOLIC MARKERS. TRANSFERRING FROM ANIMAL TESTING TO CLIMACTERIC WOMEN

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IRENE COLL RISCO

Doctoral Thesis Supervisors [Directores de la Tesis Doctoral]

Virginia A. Aparicio García-Molina
PhD
Profesora Contratada Doctora Indefinida
Universidad de Granada

Daniel Camiletti Moirón
PhD
Profesor Ayudante Doctor
Universidad de Cádiz

Doctoral Thesis Committee [Miembros del Tribunal]

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RESEARCH PROJECTS AND FUNDING

The present Doctoral Thesis was performed as a result of the following research projects:

1. Efectos de un entrenamiento combinado de fuerza y aeróbico y del tratamiento dietético sobre parámetros del síndrome metabólico en ratas genéticamente obesas. Spanish Ministry of Economy and Competitiveness (i+D+I program).
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ABSTRACT

Obesity is increasing its prevalence all over the world and is becoming an important global concern for its association with dyslipidaemia, insulin resistance, and inflammation. All these cardiometabolic markers are predictors of an increased risk of cardiovascular diseases. In this sense, an adverse cardiometabolic profile might be reversed by physical exercise, specially a combination of interval aerobic training and strength exercise (IASE).

The major aims of the present International Doctoral Thesis were: i) to examine the effects of an IASE protocol and diet on body composition and other cardiometabolic markers in metabolically obese animal subjects (Stage 1); and, ii) to extend the study of this specific training protocol to a human model at high risk of cardiometabolic alterations (i.e. climacteric women) (Stage 2).

To address these aims, five studies were conducted in the context of two research projects. Stage 1, (studies I and II) was an IASE and caloric restriction based project carried out with metabolically obese and control (lean phenotype) Zucker rats. The animals were divided in groups of 8 rats to test the influence of phenotype, the IASE protocol and caloric restriction. Stage 2 (Studies III to V) was carried out as part of the FLAMENCO (the Fitness League Against MENopause COst) project randomized controlled trial, and comprised a total of 150 climacteric women. The participants were divided into IASE and counselling group (both, n=75). Counselling consisted on four conferences about healthy diet and lifestyle. Body composition, plasma glycaemic and lipid profile and some plasma inflammatory markers were measured in both projects. Intention to treat and per protocol analyses were presented for the randomized controlled trial.

The main findings and conclusions derived from the five studies included in this Thesis were: I) In an obese phenotype of rats, the practice of an IASE protocol enhanced body composition and lipid profile, and even restore glucose concentrations to normal ranges; II) The IASE protocol improved inflammation, glycaemic profile and body composition beyond caloric restriction.

In regards to the Stage 2, the main findings were: III) An interval aerobic training combined with strength exercise protocol reduced body mass index and gynoid and

android fat mass and improved bone mineral content of the pelvis in climacteric women. Moreover, the reduction seen in gynoid and android fat mass was associated with lower pharmaceutical expenditure. IV) The interval aerobic training combined with strength exercise protocol promoted a healthier cardiometabolic profile in climacteric women. V) A greater fulfilment of the Mediterranean Dietary pattern was simply implemented through four conferences in the counselling group. Women in the exercise group increased their beer consumption, which might have been due to the social meetings after the exercise trainings.

The results of this International Doctoral Thesis enhance our understanding about how exercise positively influences cardiometabolic markers in subjects prone to metabolic alterations. Future studies should combine this IASE protocol with dietary interventions based on the Mediterranean Diet in search of better results. This Thesis closes the cycle regarding the transference of knowledge from animal testing to human research.

RESUMEN

La obesidad es un problema creciente en todo el mundo y se está convirtiendo en tema de preocupación global por su asociación con la dislipidemia, la resistencia a la insulina y la inflamación; todos ellos predictores de un mayor riesgo cardiovascular. Un perfil cardiometabólico adverso podría revertirse a través de programas de ejercicio físico, especialmente cuando se combina el ejercicio aeróbico interválico con el de fuerza (AIF).

Los objetivos generales de la presente Tesis Doctoral Internacional fueron: i) Analizar los efectos de un protocolo de ejercicio AIF y la dieta sobre la composición corporal y otros marcadores cardiometabólicos en sujetos experimentales animales metabólicamente obesos (Etapa 1); y, ii) Extender el estudio de dicho programa de ejercicio a un modelo humano con importantes alteraciones cardiometabólicas (mujeres en etapa climatérica) (Etapa 2).

Para la evaluación de dichos objetivos se realizaron cinco estudios enmarcados en dos proyectos. La Etapa 1 (estudios I y II) se desarrolló en un proyecto de experimentación animal, en ratas Zucker genéticamente obesas y de fenotipo delgado. Los animales fueron divididos en grupos de 8 para evaluar la influencia del fenotipo, el protocolo AIF y la restricción calórica. Para la Etapa II (estudios III, IV y V) se estudiaron 150 mujeres en etapa climatérica, que formaban parte del Proyecto randomizado controlado FLAMENCO (the Fitness League Against MENopause COst). Las participantes se dividieron en un grupo de entrenamiento AIF y un grupo consejos (n=75 en cada uno). El grupo consejo asistió a cuatro conferencias acerca de una dieta y estilo de vida saludables. La composición corporal y el perfil glicémico, lipídico y algunos marcadores inflamatorios plasmáticos se midieron en ambos proyectos. Los resultados del proyecto FLAMENCO se exploraron tanto en intención de tratar como por protocolo.

Los principales hallazgos y conclusiones derivados de los cinco estudios incluidos en esta Tesis fueron: I) En ratas de fenotipo obeso, la práctica de ejercicio AIF mejoró la composición corporal y el perfil lipídico, e incluso restableció las concentraciones de glucosa a rangos saludables. II) El entrenamiento AIF mejoró el perfil inflamatorio y glucémico y la composición corporal en mayor medida que la restricción calórica.
En referencia a la Etapa 2, las conclusiones principales fueron: III) El entrenamiento AIF redujo el índice de masa corporal, la masa grasa ginoide y androide y mejoró el contenido mineral óseo de la pelvis. Además, la reducción encontrada en la masa grasa se asoció

con un menor gasto farmacológico de las mujeres. IV) El entrenamiento AIF promovió un perfil cardiometabólico más saludable. V) Las cuatro conferencias dadas al grupo consejos tuvieron un impacto positivo en forma de mayor cumplimiento del patrón de Dieta Mediterranean. Las mujeres del grupo de ejercicio incrementaron su consumo de cerveza, lo cual podría deberse a los encuentros sociales que se realizaban después de los entrenamientos.

Los resultados de esta Tesis Doctoral Internacional aumentan el conocimiento sobre cómo el ejercicio afecta positivamente a los marcadores cardiometabólicos en sujetos propensos a alteraciones metabólicas. Futuros estudios deberían combinar el ejercicio AIF con intervenciones dietéticas basadas en la Dieta Mediterránea, en busca de una mejora de los resultados. Esta Tesis cierra el círculo en lo referente a la transferencia del conocimiento de modelos experimentales animales a humanos.

ABBREVIATIONS

AL, Ad Libitum

ANCOVA, Analysis of covariance

ANOVA, Analysis of variance

BMC, Bone mineral content

BMD, Bone mineral density

BMI, Body Mass Index

CI, Confidence interval

CR, Caloric restriction

CRP, C-Reactive protein

Exercise+*AL*, Exercise plus *Ad libitum*

Exercise+CR, Exercise plus caloric restriction

HDL-C, High density lipoprotein colesterol

HOMA-IR, Homeostatic model assessment for insulin resistance

IASE, Interval aerobic training combined with strength exercise

IL, Interleukin

LDL-C, Low density lipoprotein cholesterol

SD, Standard deviation

Sedentary+*AL*, Sedentary plus *Ad libitum*

sedentary+CR, Sedentary plus caloric restriction

TNF-α, Tumour necrosis factor alpha

INTRODUCTION

INTRODUCTION

Obesity is increasing prevalence all over the world, and is becoming an important global concern, mostly among high socioeconomic countries[1]. Abdominal obesity in particular, is now thought to be behind an increment of risk cardiometabolic status[2]. This abdominal obesity may contribute to the development of dyslipidaemia and insulin resistance[2,3], and is also related to increased plasma C-reactive protein (CRP), an inflammatory marker that predicts cardiovascular diseases[4]. Furthermore, adiposity indices, in addition of being an indicator for obesity, are directly associated with the incidence of metabolic syndrome[5]. In turn, the metabolic syndrome is a constellation of interrelated cardiometabolic risk factors, including raised blood pressure, fasting glucose, triglycerides and central obesity and low high-density lipoprotein cholesterol (HDL-C)[6]. The metabolic syndrome predispose the development of cardiovascular diseases[7], type 2 diabetes[8], and even cancer[9]. A healthy body composition as well as a good maintenance of the cardiovascular system helps keeping the cardiometabolic factors under control, and is key for a good quality of life.

The most non pharmacological methods employed to struggle obesity and the metabolic syndrome are caloric restriction (CR) and exercise[10–12]. The effects of *exercise* on body composition and metabolic syndrome in obese individuals have been further studied[13,14]. It is known that exercise improves cardiometabolic markers[15], and its inclusion in the lifestyle is widely recommended. In this sense, several years of research have already evidenced the need of resistance training together with aerobic training in order to achieve positive gains on cardiometabolic markers in the general population[16]. Moreover, numerous studies have demonstrated that high-intensity aerobic-anaerobic interval training amiliorates obesity and lipid profile[17–20]. Also, interval aerobic training may be more beneficial than moderate intensity exercise enhancing metabolic syndrome markers[13]. Moreover, strength training also demonstrates important metabolic effects by reducing fat and adverse plasma lipids[21–23], as well as promoting positive metabolic effects such as blood pressure and insulin resistance, and increased high-density lipoprotein cholesterol[21–23]. Consequently, the American College of Sports Medicine recommends the combination of strength training with the "classical" aerobic exercise for greater body weight loss[17]. Results of recent studies[24,25] comparing aerobic, strength and

combined aerobic-strength training encourage us to further explore the metabolic effects of this combined type of exercise.

Therefore, this Thesis focused on the study of an interval aerobic training combined with strength exercise (IASE) in the same session.

Caloric restriction is a diet based on reducing calories but maintaining healthy proportions of macronutrients[26]. In humans, CR may reduce body weight, plasma glucose, insulin and cholesterol, and systolic blood pressure[26,27]. However, many approaches to obesity treatment, largely composed of weight-loss diets, have proven no effectiveness[28]. An alternative to CR is the Mediterranean diet, known to be protective against cardiovascular diseases[29,30]. Moreover, the Mediterranean Diet is strongly associated with improvements in health status, and reductions in overall mortality, in the general population[31,32].

Finally, some studies have analysed the *combined effects of diet and exercise* and their findings support the inclusion of an exercise component in weight-loss programmes to improve metabolic fitness[10,11,26,33,34]. Of note is that the majority of studies until date have developed solely aerobic or strength training protocols[14,35–39].

In spite of these findings, there remains a critical gap between detecting positive effects of combined CR/Mediterranean diet adherence and exercise and clarifying the specific contribution of each component with regard to metabolic status.

STAGES

The present International Doctoral Thesis is structured in two stages. Stage 1 comprised the animal testing of the above-mentioned interval aerobic-strength training, and CR on cardiometabolic markers and body composition; and Stage 2, extending this specific training protocol to a human model at high risk of cardiometabolic alterations (i.e. climacteric women).

Stage 1. Interval aerobic exercise combined with strength training in metabolically obese Zucker rats

Scientific research usually starts with animal testing, especially when conducting novel protocols that have never been tested in humans, or need further scientific foundation[36,39,40]. These animal models are also useful when evaluating and analysing physiological response in specific diseases[38,41]. In this context, the genetically obese Zucker rat is an adequate animal experimental model for the study of metabolic syndrome, as presents obesity, dyslipidaemia, insulin resistance and hypertriglyceridemia[41,42].

Several studies working with animals have proven the effects of different exercise protocols[13,14,19,35,37–39], reporting that high-intensity exercise is more beneficial than moderate-intensity exercise at reducing cardiovascular disease risk in rats with the metabolic syndrome[13]. Other studies performed with the same type of rat (i.e. metabolically obese Zucker rat) have analysed different exercise protocols on various cardiometabolic markers, reporting different results. These findings include reduced weight gains[39] or improved glucose tolerance[36] and no changes in weight gains nor lipids[14].

Few studies working with animals have explored/investigated the combined effects of CR and exercise[11,40,43]. Further, exercise is usually tested in animals fed *ad libitum (AL*), a condition that does not apply for a multimodal intervention. Moreover, to the best of our knowledge, no studies have focused on the metabolic effects of an IASE protocol within the same session, and its interaction with CR in obese individuals.

Ultimately, animal testing is used in very specific and essential circumstances and its results are intended to be transferred to human studies. However very few studies go through the path of transferring the obtained results with animal testing, to human studies with the same characteristics[44,45].

In this Thesis, this stage of research was firstly conducted in order to approach the effects of aconcurrent exercise on obesity and other cardiometabolic markers, to secondarily reproduce this training in a human model in the Stage 2.

Stage 2. Interval aerobic exercise combined with strength training in climacteric women

Climacteric is a period usually comprised between ages of 40 to 65 and it incorporates the perimenopause and extends to a longer variable period before and after perimenopause[46]. It is a crucial period for women´s health due to the oestrogen loss characteristic of menopause transition[47–49], and is frequently related with weight gains and central body-fat accumulation[50]. This fact predispose these women to a higher incidence of cardiovascular diseases[49]. This transition has relevant metabolic changes associated with an increased atherogenic lipid profile[51], an increased inflammation and insulin resistance, factors known to be highly associated with greater cardiovascular disease risk[52,53]. Menopause is also associated with increased visceral adiposity[52] and increased hypertension prevalence[54] as well as a bone mass decrease[55], which increases the risk of bone fracture[56]. Therefore, improving body composition and cardiometabolic markers and preserving bone mass are desirable goals in this physiological stage. All of them might be achieved throughout exercise and healthy dietary habits.

There are controversial findings regarding the effects of different types of exercise on lipids and blood pressure at this stage, suggesting that the combination of walking (light aerobic exercise) with resistance training could improve hypertension and dyslipidaemia[57]. Furthermore, resistance training as well as moderate-to-vigorous aerobic training has shown a preventive effect on the rise of CRP in postmenopausal women[58,59]. Concerning body composition, some studies have shown improvements in some body composition variables as a result of high intensity training[49,60], moderate aerobic training[61], or resistance training[21,62]. On the other hand, there are studies that have failed to find positive changes regarding body weight[60] or fat measurements[63]. Simultaneously, there is evidence that exercise can prevent bone loss and osteoporosis[64], and higher levels of muscle strength have been associated with greater bone mineral density (BMD)[65]. A combined resistance and high-impact (or weight bearing) training protocol have been effective on enhancing BMD[66] while resistance training alone showed no effects[66,67]. Similarly, some aerobic training based protocols have shown preserved BMD[67] whereas others have failed to find differences[68]. This controversy underlines that further research is needed to understand the best exercise modality to optimise cardiometabolic markers and body composition (including bone mass) during this relevant period.

Several years of research have already evidenced the need of resistance training together with aerobic training in order to improve cardiometabolic status after menopause[69]. Therefore, a combination of aerobic and strength training could simultaneously ameliorate all the metabolic changes of this period. As far as we know, no prior study has explored the effects of IASE programme on body composition and cardiometabolic markers during climacteric.

The Mediterranean Diet is an specific dietary pattern known for its protection against cardiovascular diseases[29,30]. Despite the increasing knowledge of the benefits of a high adherence to the Mediterranean Diet, known to be cardio protective even with the appearance of risk factors[70,71], there seems to be a distancing of the Mediterranean Diet patterns among midlife women even from Mediterranean countries as is Spain[72]. Some studies suggest that less than a third of the women show a high Mediterranean Diet adherence (Mediterranean Diet Score ≥34)[73]. Furthermore, when following a training program, participants often reduce their physical activity patterns during their daily life's[74,75], and we believed that a reduction on healthy dietary patterns may also occur.

Studies are needed to explore whether climacteric women following an exercise program tend to change their lifestyle behaviour into healthier dietary habits, or on the contrary, are more permissive with their meals after burning calories through exercise. This is a relevant question in order to find the best interventional approach in this population (e.g. to register dietary habits, and to valorise the implementation of diet+exercise programs). In this manner, less unexpected changes on physiological outcomes may be found when analysing exercise groups/interventions.

AIMS/OBJETIVOS

AIMS

The overall aim of the present International Doctoral Thesis was to examine the effects of exercise and diet-based interventions on cardiometabolic markers, focusing in an interval aerobic training combined with strength exercise in the same training session. The structure of the work to fulfill the present aim was divided into two stages: *Stage 1.* when working in animal testing to analyse this novel concurrent exercise training protocol; and *Stage 2.* when conducting a transference from the animal testing to human research in climacteric women.

The specific aims of the present International Doctoral Thesis are the following:

1. To investigate the influence of this interval aerobic training combined with strength exercise protocol on body composition, physical performance, glycaemic and lipid profile and some inflammatory markers in genetically obese rats *(study I)*.

2. To study the interactions taking place between the rat's phenotype and the interval aerobic training combined with strength exercise protocol *(study I)*.

3. To compare the influence of an interval aerobic training combined with strength exercise and a caloric restriction on body composition, glycaemic, lipid and inflammatory markers in in genetically obese rats *(study II)*.

4. To explore the potential interactions taking place between the caloric restriction and the interval aerobic training combined with strength exercise protocol in in genetically obese rats *(study II)*.

5. To evaluate the influence of a 4-month of interval aerobic training combined with strength exercise protocol compared to a healthy lifestyle counselling on body composition in climacteric women *(study III)*.

6. To assess the association of body composition improvements with pharmaceutical costs in climacteric women *(study III)*.

7. To evaluate the influence of a 4-month interval aerobic training combined with strength exercise protocol compared to a healthy lifestyle counselling on plasma glucose, lipid profile, C-reactive protein and blood pressure and resting heart rate in climacteric women *(study IV)*.

8. To analyse the influence of a 4-month of interval aerobic training combined with strength exercise protocol compared to a healthy lifestyle counselling on the changes of dietary patterns and the Mediterranean Diet adherence in climacteric women. *(study V)*.

OBJETIVOS

El objetivo principal de la presente Tesis Doctoral Internacional fue profundizar en el conocimiento de los efectos del ejercicio y la dieta sobre marcadores cardiometabólicos, trabajando específicamente el entrenamiento aeróbico interválico combinado con fuerza en una misma sesión de ejercicio. El trabajo para desarrollar los presentes objetivos se dividió en dos etapas: *Etapa 1,* en la que se realizó la experimentación animal del hasta la fecha novedoso programa de entrenamiento combinado; y *Etapa 2,* realizando una transferencia del protocolo de ejercicio físico realizado en el modelo experimental animal a humanos, concretamente a mujeres en etapa climatérica.

Los objetivos específicos de esta Tesis Doctoral Internacional son los siguientes:

1. Investigar la influencia de un programa de ejercicio físico aeróbico interválico combinado con fuerza sobre la composición corporal, el rendimiento físico, el perfil glucémico y lipídico y algunos marcadores inflamatorios en un modelo de rata genéticamente obesa *(estudio I)*.

2. Estudiar las interacciones que se dan entre el fenotipo del animal y el programa de ejercicio aeróbico interválico combinado con fuerza *(estudio I)*.

3. Comparar la influencia de un programa de ejercicio aeróbico interválico combinado con fuerza con una restricción calórica, sobre la composición corporal, el perfil glucémico y lipídico y algunos marcadores inflamatorios en un modelo de rata genéticamente obesa *(estudio II)*.

4. Explorar los efectos de ambas intervenciones (i.e. restricción calórica o ejercicio) por separado, evaluando las potenciales interacciones entre ellas *(estudio II)*.

5. Evaluar la influencia de un programa de ejercicio aeróbico interválico combinado con fuerza durante 4 meses comparado con unas conferencias acerca de un estilo de vida saludable sobre la composición corporal en mujeres en etapa climatérica *(estudio III)*.

6. Analizar la asociación de las mejoras en la composición corporal con los costes farmacéuticos *(estudio III)*.

7. Determinar la influencia de un programa de ejercicio aeróbico interválico combinado con fuerza durante 4 meses comparado con unas conferencias acerca de un estilo de vida saludable sobre la glucosa, el perfil lipídico y la proteína C-reactiva en plasma, la presión arterial y la frecuencia cardíaca en mujeres en etapa climatérica *(estudio IV)*.

8. Comparar la influencia de un programa de ejercicio aeróbico interválico combinado con fuerza durante 4 meses comparado con unas conferencias acerca de un estilo de vida saludable sobre los patrones alimentarios y la Dieta Mediterránea de mujeres en etapa climatérica *(estudio V)*.

MATERIAL AND METHODS

MATERIAL AND METHODS

Stage 1. Interval aerobic exercise combined with strength training on metabolically obese rats

Effects of interval aerobic training combined with strength exercise on metabolic markers and aerobic capacity *(study I.)*, and its combination with caloric restriction (*study II*.) in Zucker rats.

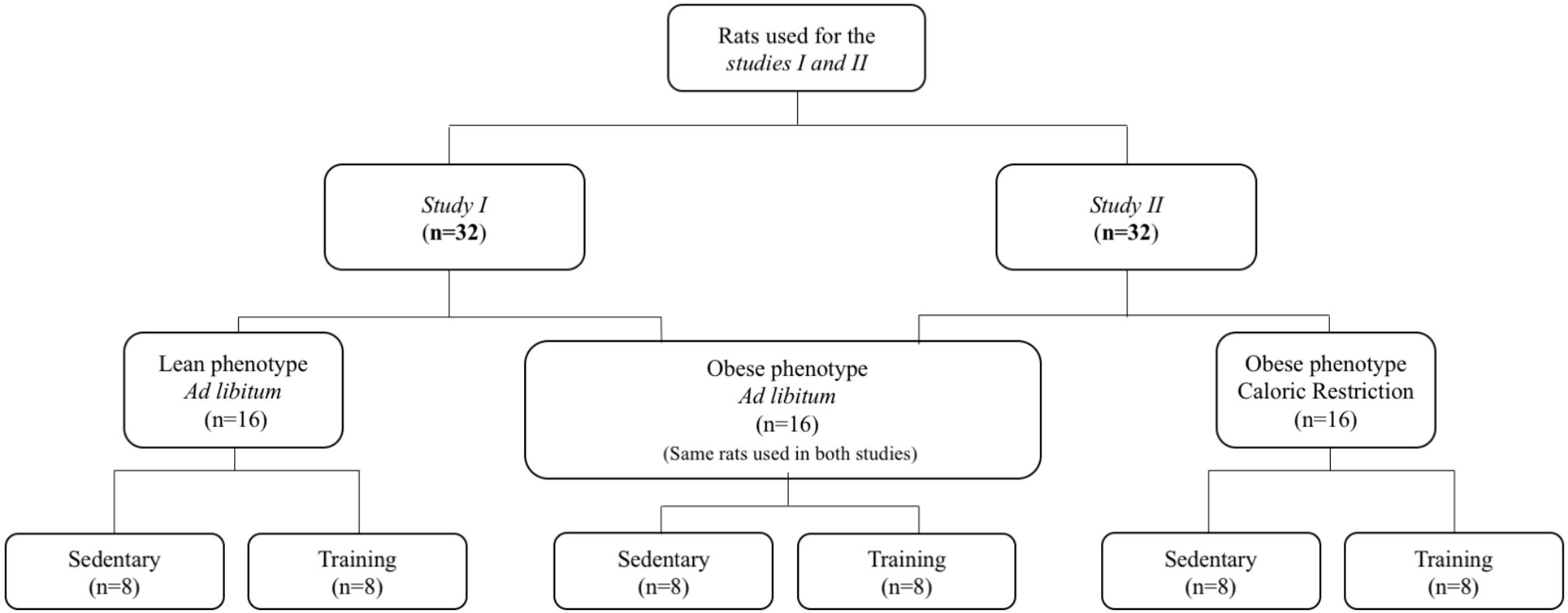
1. Study animals

Study I. Thirty-two adult male Zucker diabetic fatty rats were separated into two subgroups (n=16 each) based on their phenotype: lean or obese. Since the Zucker rat strain includes a control variety that does not present leptin resistance and consequently the obesity-derived disturbances, these rats were used as the "lean phenotype" control group. Each group was further divided into exercise or sedentary resulting in four experimental groups (lean+exercise, lean+sedentary, obese+exercise, and obese+sedentary), (all, n=8).

Study II. Thirty-two adult male Zucker diabetic fatty rats were randomly divided into CR or *ad libitum* intake groups (n=16). Each group was further divided into exercise or sedentary (n=8) resulting in four experimental groups (sedentary+CR; sedentary+*AL*; exercise+CR, and exercise+*AL*), (all, n=8).

The distribution of the animals for each study is shown in Figure 1.

Figure 1. Distribution of the rats in the studies of the present Thesis.



2. Experimental design

The animals, with an initial body weight of 165±10g (*study I*) and 162±8g (*study II*), were housed in group cages of 30*55*20 cm dimensions (4 rats per cage). The cages were located in a well-ventilated thermostatically controlled (21±2°C) room with a relative humidity ranging from 40-60% and a reverse 12h light-12h dark cycle (08:00-20:00h). Throughout the experimental period (8 weeks) all rats had free access to type-2 water (>15MΩcm).

Body weight was measured weekly for all animals and the amount of food consumed was registered daily (Ohaus® Adventurer™*Pro.* USA). At the end of the experimental period the animals were anaesthetised with ketamine-xilacine (85 mg/kg body weight of ketamine and 10 mg/kg body weight of xylazine) and euthanized by cannulation of the abdominal aorta. Quadriceps were extracted and stored at -20ºC. Blood was collected (with heparin as anticoagulant) and centrifuged at 3.000rpm for 15min to separate the plasma, which was subsequently removed and frozen in liquid Nitrogen and stored at −80°C.

The study was approved by the Animal Experimentation Ethics Committee of the University of Granada (2011-343) and all experiments were performed according to Directional Guidelines Related to Animal Housing and Care[76].

2.1. Experimental diet

Experimental diets were formulated to meet the nutrient requirements of rats based on the AIN-93M criteria[19]. In *study I* all animals were fed *AL*, in *study II*, the CR groups' food intake was 30% lower than their *AL* reference groups (e.g. exercise+CR food intake was 30% lower than exercise+*AL*).

2.2. Exercise protocol

The experimental groups trained 5 days/week following the IASE protocol. The animals ran on a motorized treadmill especially designed for rats (Panlab, Harvard apparatus. LE 8710R) and all sessions were performed during the dark cycle of the animals (active period). An electrical stimulus at the end of the treadmill forced the animals to keep

running during the whole training session. Nonetheless, the maximum discharge was set at 0.8 mV, following the manufacturer's instructions.

A week before the beginning of the experimental period, the animals were adapted to the training procedures through a low intensity running protocol, carried out daily during 20 min in the treadmill at 18 m/min. To establish the velocity for each maximal oxygen consumption a maximal incremental test was performed at the start of the experimental period and was repeated every two weeks[18]. This protocol, implemented via computer software (SeDaCom V2. Panlab. Harvard apparatus) provides an appropriate ratio of oxygen consumption and carbon dioxide production. Using the same software, maximal oxygen consumption, running time, maximal speed and total distance achieved were measured. The test ends when the animal is visibly exhausted and sited on the shock bar for >5 sec. Blood lactate concentrations from the animals' tail were measured at the end of the incremental test (Lactate Pro, Arkray, The Netherlands).

The training protocol finished three days before the end of the experimental period in order to avoid acute interferences on plasma determinations.

To establish the velocity for each maximum oxygen uptake (VO_2peak) percentage of working, a maximal incremental test was performed at the start of the experimental period and every two weeks. This protocol, implemented via computer software (SeDaCom V2. Panlab. Harvard apparatus) provides an appropriate ratio of oxygen consumption and carbon dioxide production. Using the same software, VO_2peak, running time, maximal speed and total distance achieved during the maximal test were estimated. The IASE protocol was designed and adapted from Haram et al.[13] and Kemi et al.[19] (Table 1).

All sessions consisted of 60 min of effective work. The sessions started with a 10 minutes warm-up at 40% maximal oxygen consumption, followed by the strength training consisting on eight 2-min running bouts separated by 1 min of rest where the animals ran with an inclination which was progressively increased every two weeks from 10 up to 25 degrees at a constant slow speed (20-25 cm/s, equivalent to ~30%-40% maximal oxygen consumption). The strength exercise was followed by 30 min of interval aerobic training, alternating 4 min bouts at 50-65% Maximal oxygen consumption with 3 min bouts at submaximal intensity at 65-85% maximal oxygen consumption.

The training protocol and sessions were designed and supervised by Graduates in Sport Sciences in collaboration with specialists in working with animals.

The animals of sedentary groups were touched and transported from their cages to the treadmills and back every day in order to undergo similar experimental stressful conditions as the ones of the exercise groups. The sedentary control animals also ran on the treadmill for 5 min once a week.

Table 1. Details of the interval aerobic training combined with strength exercise protocol performed by the animal training groups.

		Week 1	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Week 8**
Warm-up 10 min		40% VO_2max	40% VO_2max	40% VO_2max	40% VO_2max	40% VO_2max	40% VO_2max	40% VO_2max	40% VO_2max
Resistance Training. Eight 2-min bouts,	**Inclination**	10%	10%	15%	15%	20%	20%	25%	25%
1 min rest	**Speed**	20 cm/s	25 cm/s	20 cm/s	25 cm/s	20 cm/s	25 cm/s	20 cm/s	25 cm/s
Interval Aerobic Training 30 min		4 min at 50% VO2max alternated with 3 min at 65%	4 min at 55% VO2max alternated with 3 min at 65%	4 min at 55% VO2max alternated with 3 min at 70%	4 min at 60% VO2max alternated with 3 min at 70%	4 min at 60% VO2max alternated with 3 min at 75%	4 min at 65% VO2max alternated with 3 min at 75%	4 min at 65% VO2max alternated with 3 min at 80%	4 min at 65% VO2max alternated with 3 min at 85%

3. Measurements

3.1 Body composition analysis

Determinations of body composition were assessed by means of a whole-body composition analyser based on magnetic resonance (EchoMRI™; EchoMedical Systems, Houston TX). This analyser estimates fat tissue (g), lean tissue (g), free water (mL) and total body water (mL) in live animals.

3.2. Biochemical analyses

Plasma total cholesterol, low density lipoprotein-cholesterol (LDL-C), HDL-C, phospholipids, triglycerides and glucose were measured using an autoanalyzer (Hitachi-Roche p800, F. Hoffmann-La Roche Ltd. Switzerland). The cytokines tumour necrosis factor alpha (TNF-α) and interleukin (IL)-1 and IL-10 were measured with the rat kit (Milliplex. MAP kit; Millipore) and calibrated with Luminex 100/200 calibration kit. Adiponectin was measured with the Sandwich Rat Adiponectin ELISA kit. Plasma leptin and insulin concentrations were measured using the panel Rat Bone for rats (Milliplex. MAP kit; Millipore) and Luminex 200TM. The homeostatic model assessment for insulin strength was calculated using the formula [fasting insulin (μIU/mL) x fasting glucose (mg/dL)]/405.

On week 8, a 12 h urine sample from each animal was collected. Prior to recollection rats were allocated in individual metabolic cages designed for the separate collection of faeces and urine. Urine volumes were recorded and urine glucose was measured using an autoanalyzer (Hitachi-Roche p800, F. Hoffmann-La Roche Ltd. Switzerland).

Forty-eight hours prior to the end of experimental period, an oral glucose tolerance test was performed following the protocol described by Prieto PG[77]. Blood glucose concentration from the animals' tail was recorded at periods 0, 15, 30, 90 and 120 min (Breeze®2, Bayer) in order to calculate the area under the curve. Homeostatic model assessment for insulin resistance (HOMA-IR) was calculated.

3.3. Quadriceps' Nitrogen determination

Quadriceps were dried to constant weight in an oven at 105±1ºC. Total nitrogen (N) of the quadriceps was determined according to Kjeldahl's method. Crude protein was

calculated as N x 6.25.

Stage 2. Interval aerobic exercise combined with strength training on climacteric women

Effects of interval aerobic training combined with strength exercise on body composition (*study III)*, cardiometabolic status (*study IV*) and dietary behaviour (*study V*) in perimenopausal women. Findings from the FLAMENCO Project Randomised Controlled Trial

The complete methodology of the FLAMENCO study is published elsewhere [78]. This trial was registered at ClinicalTrials.gov (identifier: NCT02358109).

1. Participants

Sample size calculations were made for the primary aim of the FLAMENCO randomised controlled trial, which was a cost-effectiveness analysis of exercise on menopausal costs. In this randomised controlled trial, a total of sample of 214 perimenopausal women (age range 45-60 years old) from Granada (Southeast Spain) were recruited through primary care centres and press releases published in local newspapers and social media. All women signed an informed consent to take part in the present study. Inclusion and exclusion criteria are detailed elsewhere in Table 2.

2. Randomization

After the baseline assessments, a total of 150 women voluntarily participated and were randomised into either a counselling (n=75) or exercise (n=75) group. A computer-generated simple randomisation sequence was created to allocate the participants to either the exercise or counselling group (1:1). The randomisation sequence was prepared by a member of the research team with no clinical involvement in the trial.

2.1.Follow-up

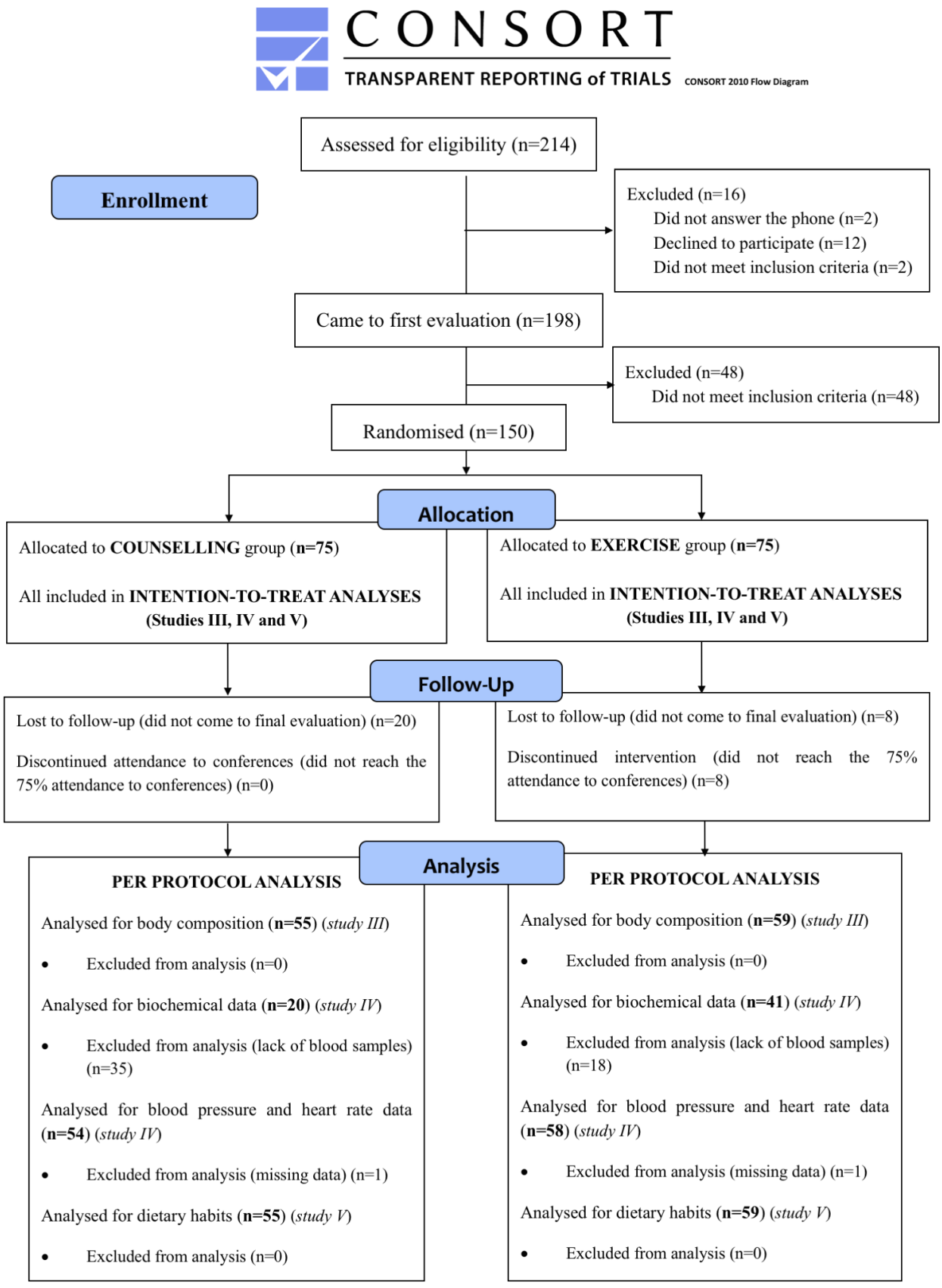
Of the 150 women that were randomised into counselling (n=75) and exercise (n=75) groups used on the intention-to-treat analyses, 20 and 8 of them dropped out the follow-up in counselling and exercise groups, respectively. A total of 8 women did not attend 75% of the exercise sessions. Thus, the total number of women used for per-protocol analyses was n=55 for counselling group and n=59 for exercise group. However, loss of

samples and lack of data are also taken into account when performing per-protocol analyses, hence the total *n* varies depending on the variables measured and is detailed in the flow chart of the studies' participants (Figure 2).

Table 2. Inclusion and exclusion criteria for the FLAMENCO randomised Controlled Trial.

Inclusion criteria:
1) being between 45 and 60 years old
2) not having severe somatic or psychiatric disorders, or diseases that prevent physical exercise (Answer "no" to all questions on the Physical Activity Readiness Questionnaire (PAR-Q)).
3) not being engaged in regular physical activity >20 minutes on >3 days/week in the last 3 months
4) being able to ambulate without assistance
5) being able to communicate
6) being capable and willing to provide informed consent
Exclusion criteria:
1) suffering from acute or terminal illness
2) having suffered a major cardiovascular event (i.e. myocardial infarction, angina, or stroke) in the past 6 months
3) inability to ambulate
4) suffering from an unstable cardiovascular disease or other medical condition
5) having had upper or lower extremity fracture in the past 6 months
6) unwillingness to complete the study requirements
7) presence of neuromuscular disease or drugs affecting neuromuscular function

Figure 2. Flow chart of participants in the studies III, IV and V.



3. Experimental design

The same group of researchers assessed socio-demographic and clinical characteristics as well as body composition on a single day and in this order. Socio-demographic (including age, educational, and marital status, among others), clinical, and other health-related information was collected using a self-reported questionnaire. This anamnesis also included questions regarding their clinical history. The use of hormone therapies, intake of osteoporosis drugs and suffering from diabetes or hypertension was registered. The Blatt-Kupperman menopausal index[79] was employed to assess menopause symptomatology.

All measurements were taken at baseline (within one week before the intervention) and after four months (after the end of the intervention period).

This study was approved by the Ethics Committee of the "Hospital Virgen de las Nieves" (Granada, Spain). The trial was registered in clinicaltrials.gov (NCT02358109).

These are secondary analyses from the FLAMENCO Project randomised controlled trial[78].

3.1. Exercise intervention

The women randomised into the exercise group participated in a four-month (60 minutes/session, 3 days/week) primary care-based exercise intervention consisting in a moderate-vigorous intensity concurrent exercise programme (aerobic and resistance). The exercise intervention followed the training standards by the American College of Sports Medicine for adults[80].

Each exercise session included a 10-minute warm-up period with walks and mobility exercises, followed by the main part, with a 40-minute duration, which varied across week days (i.e. 3 different models of session). The sessions finished with a 10-minute cool-down period of stretching and relaxation exercises. The first session of the week involved circuit training including resistance exercises in a stepped progression throughout the programme. The second session of the week included balance oriented activities (position changes, monopodal and bipodal stances etc.) and dancing (aerobic exercises). The third session of the week combined aerobic, resistance, and coordination exercises within the same session[78]. The ratings of perceived exertion were monitored using the Borg 6-20

Ratings of Perceived Exertion (RPE)[81] scale during all the sessions. The intensity (expressed as RPE) ranged from 12 to 16.

3.2. Counselling group

The women in the counselling group did not participate in the exercise sessions, and they were requested to continue their daily activities. Even so, as a healthy diet and increasing physical activity levels and exercise have proven beneficial effects, the research team undertook four conferences (one each month) addressing different topics: 1) benefits of exercise for longevity, prevention, and treatment of diseases; 2) benefits of the Mediterranean diet and nutritional education; 3) ergonomic advice and exercises to do at home (e.g. resistance training); and 4) strategies to increase daily physical activity levels. Due to the importance of this type of intervention, the "control" group was renamed "counselling" group. The conferences were also used to maintain their fidelity until the end of the programme. Only counselling group was invited to these conferences.

4. Measurements

4.1. Anthropometry and body composition

A portable eight-polar tactile-electrode impedanciometer (InBody R20, Biospace, Seoul, Korea) was used to measure body weight. Height (cm) was measured using a stadiometer (Seca 22, Hamburg, Germany). Lean mass; fat mass, visceral adipose tissue, gynoid fat mass, android fat mass; total BMD, BMD of lumbar spine and pelvis; total bone mineral content (BMC) and BMC of pelvis were measured using a dual-energy X-ray absorptiometry (DXA) device (Hologic Discovery QDR, Nasdaq: HOLX). Body mass index (BMI) as weight (kg) divided by height (m) squared was calculated.

4.2. Plasma glucose, lipid profile, and C-reactive protein

Our team collected venous blood samples in standardised fasting conditions in the women's primary care centre. Then, the samples were centrifuged at 1750 rpm for 10 minutes at 4°C in a refrigerated centrifuge (GS-6R Beckman, Fullerton, CA, USA) to separate plasma from formed elements. They were then analysed in the laboratory using

an autoanalyser (Hitachi-Roche p800, F. Hoffmann-La Roche Ltd. Switzerland). Plasma glucose, total cholesterol, LDL-C, HDL-C, CRP, and triglycerides were measured.

4.3. Blood pressure and resting heart rate

Systolic and diastolic blood pressure as well as resting heart rate were measured twice (2 minutes in-between) with a blood pressure monitor (Omron Health Care Europe B.V. Hoolddorp) after the women sat for at least 5 minutes. The lowest value of both assessments was used for the analyses.

4.4. Mediterranean Diet Score

The Mediterranean Diet Score is an index created to evaluate the degree of adherence to the traditional Mediterranean dietary pattern[82]. It consists of 11 items (non-refined cereals, potatoes, fruits, vegetables, legumes, fish, olive oil, red meat and derivates, poultry, full fat dairy products, and alcohol) and the total score ranges from 0–55, with higher scores indicating greater adhesion to the Mediterranean dietary pattern.

4.5. *Pharmaceutical costs*

The pharmaceutical consumption of each patient was obtained through the medical history from the DIRAYA system, used by the Public Health System of Andalusia [83].

The cost of medication was calculated with the 2015 prices in Spain. The consumption cost of prescribed pharmaceuticals for each patient before and during the study was calculated based on the prices, prescribed dose, and schedule of administration. To take into account all possible medication changes over the four-month intervention, data on medication in both groups was recorded at the initial, middle, and final phase of the study.

4.6. Physical activity levels

A triaxial accelerometer GT3X+ (Actigraph, Pensacola, FL, USA) was used to measure activity counts (rate of 30 Hz and stored at an epoch length of 60 s). The women wore the device on the hip near their centre of gravity, underneath clothing, and secured with an elastic belt.

Physical activity was measured the week before starting the exercise intervention and the week after finishing it, 4 months later. It was recorded up to 9 days, starting from the day that the women received the accelerometers until the day they were instructed to return

the devices. The number of minutes of moderate-vigorous physical activity bouts per week was also calculated. Bouted moderate-vigorous physical activity was defined as a period of ≥10 consecutive minutes spent in that behaviour. Women were classified as meeting or not the American College of Sports Medicine guidelines for adults [80], (>150 minutes/week of bouted moderate-vigorous physical activity) Data download, reduction, cleaning, and analyses were performed using ActiGraph software (ActiLife v. 6.11.9).

Statistical analyses

Results are presented as mean and standard deviation. All the variables were checked for normality of distribution before the analyses.

The statistical analyses were conducted with the Statistical Package for Social Sciences (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp). The statistical significance was set at $P<0.05$.

The statistical approach undertaken to accomplish the aims of the present International Doctoral Thesis is presented below and is summarized in Table 3.

Stage 1. Interval aerobic exercise combined with strength training on metabolically obese rats

Study I. Effects of interval aerobic training combined with strength exercise on metabolic markers and aerobic capacity.

The effects of the rat phenotype and IASE (sedentary vs. exercise) on body composition, aerobic capacity markers, lipid and glycaemic profile and cytokines were analysed by two-way factorial analysis of variance (ANOVA), with rat's phenotype and exercise as fixed factors. Two-ways interaction terms were introduced into the models to test interactions between phenotype*exercise. A significant *P* value indicates that there are differences at least between two of the groups. *Cohen's d* and its exact 95% confidence interval were used in all the comparisons to estimate the standardized effect size. The "phenotype effect size" has been calculated combining both groups of the same phenotype compared to the other two groups (i.e. lean+sedentary and lean+exercise vs. obese+sedentary and obese+exercise). The "exercise effect size" has been calculated combining both groups of exercise compared to the two sedentary groups (i.e. lean+sedentary and obese+sedentary vs. lean+exercise and obese+exercise). Values of Cohen's *d* ~0.2, ~0.5 and ~0.8 were considered to represent small, medium and large effects, respectively.

Additionally, Bonferroni's adjustment was made on oral glucose tolerance test results to

identify between which groups the differences were significant (e.g. obese+sedentary vs. lean+exercise group).

Study II. Interval aerobic training combined with strength exercise improves metabolic markers beyond caloric restriction (study II.) in Zucker rats.

The effects of caloric restrction and IASE were analysed by two-way factorial analysis of variance (ANOVA), with CR and exercise as fixed factors. Two-way interaction terms were modelled to test for interaction effects between CR and exercise. Significant *P* values indicate statistical differences in metabolic profile between at least two of the groups. *Cohen's d* and its exact confidence interval were used in all the comparisons to estimate the standardized effect size.

A clustered adverse body composition (z-score) as the mean of the standardized scores [(value-mean)/standard deviation] of fat mass and lean mass (inverted) was created (Figure 3A). Similarly, a clustered adverse lipid profile as the mean of the standardized scores of plasma triglycerides, LDL-C, phospholipids and inverted HDL-C (Figure 3B), a clustered glycemic profile as the mean of the standardized scores of fasting glucose, area under the curve after oral glucose tolerance test and HOMA-IR (Figure 3C) and a clustered overall cardiometabolic risk including body composition, lipid and glycemic components (Figure 3D). Finally, a clustered exercise capacity index including aerobic capacity markers and quadriceps protein content was created (Figure 4). All the clustered risks components were compared across groups by ANOVA.

Additionally, post-hoc multiple comparisons with the Bonferroni's correction were applied on figures to examine the specific difference between each group (e.g. sedentary+CR vs exercise+*AL*).

Stage 2. Interval aerobic exercise combined with strength training on climacteric women

Study III. Effects of interval aerobic training combined with strength exercise on body composition changes

Descriptive statistics are shown as mean (standard deviation, SD) for quantitative variables, and number of women (%) for categorical variables. The effects of a four-month exercise intervention on lean, fat, and bone mass were assessed with linear regression on the intention-to-treat population. The changes (post−pre) in body composition were included as dependent variables in separate models and the group (counselling=0 and exercise=1) as an independent variable. The baseline value was a potential cofounder for all variables. For bone-related parameters, age, fat and lean mass, and hormone therapy were considered and finally included as potential cofounders, as the between-group differences changed importantly after their inclusion. Furthermore, "meeting the minimum physical activity recommendations" was considered as a new cofounder, but the results remained the same, and it was not included. In addition, the per-protocol analyses including the counselling group participants (all attended at least 75% of the conferences), and the exercise group participants (with ≥75% attendance at the exercise programme) were also undertaken to account for the potential clinical efficacy of the exercise programme. A multiple imputation was made for the missing values in order to perform intention-to-treat analyses.

Linear regression analysis was used to assess the association of the change in body composition (independent variable) with the change in pharmaceutical cost (dependent variable).

Study IV. Effects of interval aerobic training combined with strength exercise on cardiometabolic status

Descriptive statistics are shown as mean (standard deviation, SD) for quantitative variables, and number of women (%) for categorical variables. A multiple imputation was made for the missing values in order to perform intention-to-treat analyses. The

effects of the 4-month exercise intervention and counselling group on plasma glucose, lipid profile, CRP concentrations, blood pressure, and heart rate were assessed in the per-protocol analysis including the exercise group participants (with ≥75% attendance at the exercise programme) and the counselling group participants (all attended at least 75% of the conferences). Changes (post-pre values) in all the outcome variables were calculated. Subsequently, the changes (post-pre) of these outcomes were included in the linear regression analyses as dependent variables in separate models, whereas the group (counselling=0 and exercise=1) was included as an independent variable. Different potential confounders were employed in these analyses. Potential confounders that could overestimate the results or influence the effects of exercise were analysed, and the most relevant were finally included. Depending on the model, the data provided was unadjusted for any confounder, adjusted for the baseline value of each variable, or adjusted for the baseline values and Mediterranean Diet Score. Because of the important loss of blood samples due to a problem that could not be prevented at the hospital where the samples were collected, another analysis assessing the 150 women based on the assigned intervention at the time of randomisation, regardless of adherence and missing data (i.e., intention-to-treat), with a multiple imputation made for the missing values, was added.

Study V. Influence of participating in a group exercise program on dietary behavior. The after-training beer phenomena. Findings from the FLAMENCO Project

Descriptive statistics are shown as mean (standard deviation, SD) for quantitative variables, and number of women (%) for categorical variables. A multiple imputation was made for the missing values in order to perform intention-to-treat analyses. The differences between counselling or exercise group on dietary patterns were analysed by linear regression. The changes (postest-pretest) of these outcomes were included in the linear regression analyses as dependent variables, and the group (counselling=0 and exercise=1) as an independent variable. Two separate models were included: Model one was unadjusted; model two was adjusted for baseline values.

Table 3. Summary table of the methods and statistical approach of each study of the present International Doctoral Thesis.

		Main variables		
	Design	**Independent variables**	**Dependent Variables**	**Statistical analyses**
Stage 1. Animal testing				
Study I	Longitudinal	IASE and Phenotype	Body composition, physical performance, glycaemic and lipid profile and inflammatory markers	- Two-way factorial analysis of the variance (A with two-ways interaction (phenotype*exercis -Bonferroni's adjustment was made in the oral tolerance test
Study II	Longitudinal	IASE and caloric restriction	Body composition, glycaemic and lipid profile, inflammatory markers, quadriceps' nitrogen	- Two-way factorial analysis of the variance (A with two-ways interaction restriction*exercise) -Z scores for clustered adverse cardiometaboli markers - Bonferroni's adjustment was made between group

Stage 2. Climacteric women

Study III	Longitudinal	IASE and counselling	Body composition and pharmaceutical costs.	- Multiple imputation for missing values independent variables Linear regression with changes (post-pre va independent variables) as dependent v (baseline values as cofo - Linear regression with changes in body com as independent variables and pharmaceutic changes as dependent variables.
Study IV	Longitudinal	IASE and counselling	Plasma lipid profile, glucose and C-reactive protein	- Multiple imputation for missing values independent variables Linear regression with changes (post-pre va independent variables) as dependent v (baseline values as cofounders).
Study V	Longitudinal	IASE and counselling	Dietary habits and the Mediterranean Diet Score	- Multiple imputation for missing values independent variables Linear regression with changes (post-pre va independent variables) as dependent v (baseline values as cofounders).

IASE, Interval Aerobic training combined with Strength Exercise; ANOVA, analysis of the variance.

RESULTS

RESULTS

The results of each individual study comprising the present International Doctoral Thesis are presented below.

Stage 1. Interval aerobic exercise combined with strength training on metabolically obese rats

Study I. Effects of interval aerobic training combined with strength exercise on body composition, glycaemic and lipid profile and aerobic capacity of obese rats

(*Journal of Sport Sciences*. 2015. Vol:34; 15. Pag. 1452-1460. http://dx.doi.org/10.1080/02640414.2015.1119296)

1.1. Food intake, body weight and body composition

The effects of the rat's phenotype and exercise on final body weight, food intake and body composition are shown in Table 4. Body weight was lower in the exercise compared to the sedentary groups of same phenotype ($P<0.01$). Exercise groups showed reduced fat mass compared to the sedentary groups for both phenotypes ($P<0.001$). Lean body mass was increased in both exercise groups compared to their sedentary counterparts ($P<0.05$). Obese+sedentary rats showed the highest fat mass whereas exercise interacted at reducing it ($P<0.004$). An exercise*phenotype interaction was also found on food intake that increased by 17% when exercise was introduced to the lean phenotype while only increased by 8% when it was introduced to the obese phenotype ($P<0.001$).

1.2. Aerobic capacity

The effects of the rat's phenotype and exercise on aerobic capacity markers are shown in Table 5. Post-maximal incremental test blood lactate was lower in the lean compared to the obese groups ($P<0.001$). Maximal oxygen consumption was higher in the exercise compared to the sedentary groups ($P<0.001$). The total running time, maximal speed and the distance covered in the incremental test by the exercise groups were higher than the sedentary groups for both phenotypes (all, $P<0.001$). An exercise*phenotype interaction was found in post-maximal incremental test blood lactate concentrations. The obese+sedentary group obtained the highest lactate concentrations and exercise decreased it by 34% in the obese phenotype while increased it by 6% in the lean phenotype (interaction $P<0.01$). Significant interactions were also found in maximal oxygen consumption that was increased by 63% when the exercise was introduced to the obese phenotype, while only increased by 13% in the lean phenotype (interaction $P<0.001$). Obese+sedentary rats obtained the lowest values for total running time, maximal speed and distance covered in the maximal treadmill tests and exercise interacted by increasing these levels (all interactions, $P<0.001$).

1.3. Plasma lipid profile

The effects of the rat's phenotype and exercise on plasma triglycerides, low-density lipoprotein-cholesterol, high-density lipoprotein-cholesterol and phospholipids are shown in Table 6. Plasma triglycerides, low-density lipoprotein-cholesterol, high-density lipoprotein-cholesterol and phospholipids were lower in the lean compared to the obese groups (all, $P<0.001$). Plasma low-density lipoprotein-cholesterol and total cholesterol were lower in the exercise compared to the sedentary groups for both phenotypes (all, $P<0.001$). A significant decrease of plasma phospholipids was found in the exercise compared to the sedentary groups ($P<0.01$). The obese+sedentary group obtained the highest values for low-density lipoprotein-cholesterol, whereas exercise interacted reducing these values ($P<0.01$). Exercise increased high-density lipoprotein-cholesterol levels in the lean group and decreased these levels in the obese group (interaction $P<0.05$).

1.4. Plasma glycaemic profile

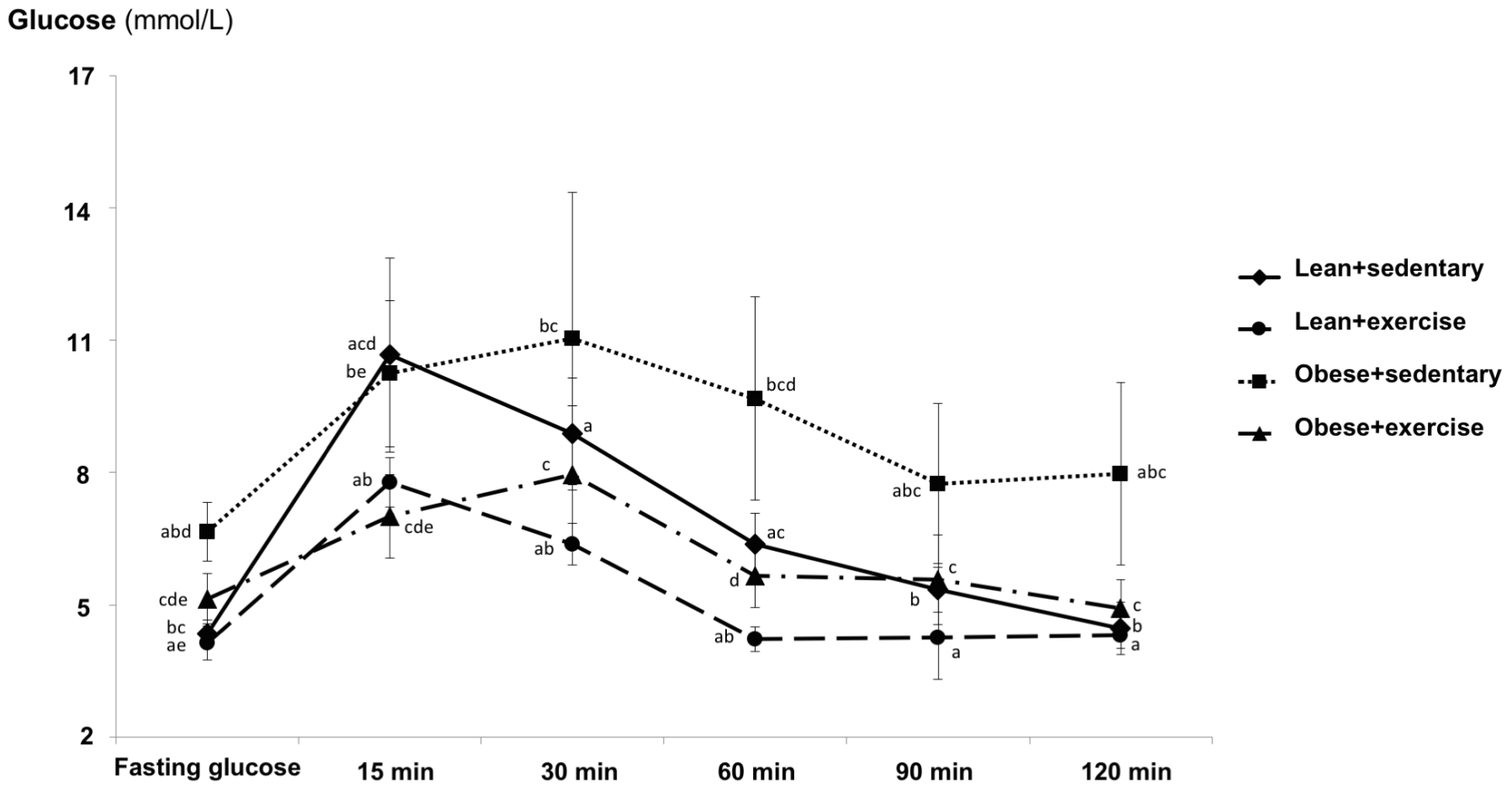
The effects of the rat's phenotype and exercise on glycaemic profile are shown in Table 6. Figure 3 additionally shows fasting and postprandial glucose after the oral glucose tolerance test**.** Fasting glucose and insulin were higher in the obese compared to the lean phenotype (both, $P<0.001$); and in the sedentary compared to the exercise groups (both, $P<0.001$). Urine glucose was also higher in the obese compared to the lean phenotype ($P<0.01$). The obese and sedentary groups obtained an increased homeostatic model assessment index than their respective lean and exercise groups (both, $P<0.001$).

Regarding the oral glucose tolerance test, postprandial blood glucose at 15, 30, 60 and 90 min was lower in the exercise compared to the sedentary groups for both phenotypes (all, $P<0.01$). Some exercise*phenotype interactions were found for plasma insulin, homeostatic model assessment index (both, $P<0.001$), fasting glucose, and the area under the curve after the oral glucose tolerance test (both, $P<0.01$), showing a higher effect of interval aerobic training combined with strength exercise on improving glycaemic profile markers in the obese than in the lean group. Finally, obese+sedentary rats obtained the highest fasting and postprandial glucose after 120 min but exercise reduced these levels (both interaction $P<0.01$).

1.5. Plasma inflammatory markers

Tumour necrosis factor alpha was increased in both exercise compared to sedentary groups ($P<0.01$). In addition, obese rats showed lower levels of adiponectin than lean rats ($P<0.001$). Exercise reduced levels of IL-1 and IL-10 in the lean group but increased these levels when exercise was introduced in the obese group (both interactions $P<0.05$).

Figure 3. Fasting and postprandial glucose at 15, 30, 60, 90 and 120 min for lean and obese sedentary and trained rats.



[a,b,c,d,e] Common superscript indicates a significant difference ($P<0.05$) between groups with the same letter. Pairwise comparisons were performed with Bonferroni's adjustment.

Table 4. Effects of the interval aerobic training combined with strength exercise protocol on final body weight, food intake and body composition for lean and obese rats.

	Lean+ sedentary	Lean+ exercise	%*	Obese+ sedentary	Obese+ exercise	%*	*P* Phenotype	*P* Exercise	*P* Exercise x Phenotype	Effect Size Phenotype†[a]	Effect Si Exercise
Final body weight, g	318 (16.1)	282 (12.2)	-11.4	462.2 (33.0)	438 (28.3)	-5.2	<0.001	0.003	0.518	-6.3 (-8.8, -3.7)	1.3 (0.2, 2
Food intake, g/day	20.5 (0.2)	23.9 (0.1)	16.6	25.4 (0.2)	27.5 (0.2)	8.3	<0.001	<0.001	<0.001	-24.0 (-33.1, -15.0)	-15.6 (-21.4,
Fat mass, g	20.0 (5.2)	15.0 (3.3)	-25.0	218 (13.3)	184 (21.6)	-15.4	<0.001	<0.001	0.004	-14.5 (-20.0, -9.0)	1.8 (0.5, 3
Lean mass, g	241 (36.4)	244 (27.6)	1.5	186 (29.1)	204 (19.1)	9.5	<0.001	0.025	0.144	1.7 (0.5, 2.9)	-0.4 (-1.4,
Total body water, ml	114 (8.1)	110 (8.1)	-3.3	164 (12.4)	174 (9.1)	6.3	<0.001	0.375	0.063	-0.6 (-8.5, -3.5)	-0.3 (-1.4,

Values expressed as mean (standard deviation). † Effects size statistics are expressed as Cohen's d(95% exact confidence interval). * Percentage of difference between the sedentary and exercise groups was computed as ((exercise-sedentary)/sedentary)x100. [a]Effect size corresponds to lean+sedentary and lean+exercise versus obese+sedentary and obese+exercise. [b]Effect size corresponds to lean+sedentary and obese+sedentary versus lean+exercise and obese+exercise.

Table 5. Effects of the interval aerobic training combined with strength exercise protocol on aerobic capacity markers for lean and obese rats.

	Lean+ sedentary	**Lean+ exercise**	**%***	**Obese+ sedentary**	**Obese+ exercise**	**%***	***P* Phenotype**	***P* Exercise x Phenotype**	***P* Exercise**	**Effect Size†[a] Phenotype**	**Effect Size†[b] Exercise**
Exercise Lactate, mmol/L	5.5 (1.8)	5.8 (3.7)	5.5	12.5 (3.7)	8.3 (4.0)	-33.6	<0.001	0.002	0.278	-1.4 (-2.6, -0.2)	0.6 (-0.5, 1.7)
VO_2max, mL/min/kg$^{0.75}$	14.2 (2.5)	16.1 (5.9)	13.4	14.7 (5.9)	24.0 (4.9)	63.3	<0.001	<0.001	<0.001	-0.9 (-2.0, 0.2)	-1.2 (-2.3, 0.0)
Running Time, min	15.6 (1.2)	20.3 (4.2)	30.1	12.2 (1.2)	16.6 (1.4)	36.1	<0.001	0.455	<0.001	1.7 (0.5, 2.9)	-2.1 (-3.4, -0.8)
Maximal Speed, cm/s	58.4 (5.6)	78.3 (17.1)	34.1	44.2 (4.7)	63.3 (7.1)	43.2	<0.001	0.435	<0.001	1.6 (0.4, 2.8)	-2.1 (-3.4, -0.8)
Distance, cm	19836 (3932)	40604 (18533)	104.7	11456 (2182)	23741 (5379)	107.2	<0.001	0.428	<0.001	1.5 (0.3, 2.7)	-1.9 (-3.2, -0.6)

Values expressed as mean (standard deviation). † Effects size statistics are expressed as Cohen's d (95% exact confidence interval). VO_2max, maximal oxygen consumption. * Percentage of difference between the sedentary and training groups was computed as ((exercise-sedentary)/sedentary)x100. . [a]Effect size corresponds to lean+sedentary and lean+exercise versus obese+sedentary and obese+exercise. [b]Effect size corresponds to lean+sedentary and obese+sedentary versus lean+exercise and obese+exercise.

Table 6. Effects of the interval aerobic training combined with strength exercise protocol on lipid profile, glycaemic profile and inflammatory markers for lean and obese rats.

Lipid profile	**Lean+ sedentary**	**Lean+ exercise**	**%***	**Obese+ sedentary**	**Obese+ exercise**	**%***	***P* Phenotype**	***P* Exercise**	***P* Exercise x Phenotype**	**Effect Size †[a] Phenotype**	**Effect Size †[b] Exercise**
Triglycerides, mmol/L	0.83 (0.59)	0.45 (0.12)	-46.1	5.97 (1.17)	6.27 (1.44)	5.0	<0.001	0.906	0.335	-5.7 (-8.1, -3.3)	0.1 (-1.0, 1.1)
LDL cholesterol, mmol/L	0.16 (0.03)	0.11 (0.02)	-31.7	1.11 (0.28)	0.51 (0.29)	-53.5	<0.001	<0.001	0.001	-3.3 (-5.0, -1.7)	2.0 (0.7, 3.2)
HDL cholesterol, mmol/L	0.50 (0.09)	0.63 (0.11)	26.0	1.78 (0.21)	1.66 (0.17)	-7.8	<0.001	0.914	0.019	-7.8 (-10.8, -4.7)	0.0 (-1.0, 1.1)
Total cholesterol, mmol/L	2.03 (0.19)	1.63 (0.20)	-20	5.80 (0.66)	4.86 (0.26)	-16.2	<0.001	<0.001	0.062	-10 (-13.8, -6.1)	2.0 (0.7, 3.3)
Phospholipids, mmol/L	1.75 (0.29)	1.29 (0.12)	-26.4	5.05 (0.47)	4.65 (0.43)	-8.0	<0.001	0.002	0.818	-9.5 (-13.2, -5.8)	1.3 (0.1, 2.5)
Glycaemic profile											
Blood fasting glucose, mmol/L	4.9 (0.30)	4.7 (0.36)	-3.9	7.1 (0.62)	5.7 (0.53)	-19.8	<0.001	<0.001	0.001	-3.3 (-4.9, -1.7)	1.8 (0.5, 3.0)
Area under the curve	3836 (1080)	1709 (378)	-55.4	6190 (1812)	2252 (751)	-63.6	0.002	<0.001	0.038	-1.4 (-2.6, -0.2)	2.8 (1.3, 4.2)
Urine volumen, ml/12 h	3.0(0.8)	3.8 (1.2)	26.7	7.1(3.2)	5.9 (1.8)	-16.9	<0.001	0.682	0.097	-1.6 (-2.8, -0.4)	0.1 (-0.9, 1.2)
Urine glucose, mmol/L/12 h	3.0 (2.04)	2.2 (0.65)	-25.6	4.0 (1.75)	3.7 (1.88)	-7.4	0.006	0.200	0.569	-0.8 (-1.9, 0.3)	0.3 (-0.7, 1.4)

Urine glucose, (mmol/L)/ml/12 h	0.94 (0.81)	0.69(0.47)	-26	0.67 (0.65)	0.85 (0.44)	21.4	0.800	0.776	0.214	0.1 (-1.0, 1.1)	0.1 (-1.0, 1.1)
Insulin, pmol/L	8.90 (2.96)	8.73(1.57)	-1.9	38.72 (6.85)	21.82 (4.42)	-43.8	<0.001	<0.001	<0.001	-5.0 (-7.2, -2.9)	2.1 (0.8, 3.4)
HOMA-IR	0.3 (0.1)	0.2(0.1)	-33.3	6.1 (1.9)	1.6 (0.6)	-73.8	<0.001	<0.001	<0.001	-4.1 (-5.9, -2.2)	3.1 (1.5, 4.6)
Leptin, µg/L	8.31 (10.49)	3.88(4.78)	-53.4	574.24 (133.68)	51.94 (28.29)	21.5	0.121	0.169	0.166	-5.3 (-7.6, -3.1)	5.0 (2.9, 7.2)
Inflammatory markers											
IL 1, pg/mL	150 (162.6)	45.7 (67.3)	-69.6	60.7 (40.8)	98.9 (54.2)	62.9	0.861	0.333	0.049	0.2 (-0.8, 1.3)	0.4 (-0.7, 1.5)
IL 10, pg/mL	134 (96.5)	99.2 (111)	-26.3	84.7 (42.2)	206.5 (93.3)	143.8	0.920	0.119	0.023	-0.3 (-1.4, 0.7)	-0.5 (-1.6, 0.6)
TNF-α, pg/mL	0.8 (0.5)	1.3 (1.1)	62.5	0.2 (0.2)	0.4 (0.3)	100.0	0.006	0.288	0.788	1.3 (0.1, 2.4)	-0.6 (-1.7, 0.4)
Adiponectin, µg/mL	0.2 (0.05)	0.20 (0.02)	-3.6	0.14 (0.03)	0.17(0.04)	43.2	<0.001	0.311	0.084	1.5 (0.3, 2.6)	-0.3 (-1.4, 0.7)

Values expressed as mean (standard deviation). † Effects size statistics are expressed as Cohen's d (95% exact confidence interval). [a]Effect size corresponds to lean+sedentary and lean+exercise versus obese+sedentary and obese+exercise. [b]Effect size corresponds to lean+sedentary and obese+sedentary versus lean+exercise and obese+exercise. LDL, low-density lipoprotein; HDL, high-density lipoprotein; A.U., Arbitrary Units; HOMA-IR, Homeostasis Model Assessment- Insuline Resistance; IL, interleukin; TNF, tumor necrosis factor. * Percentage of difference between the sedentary and training groups was computed as ((exercise-sedentary)/sedentary)x100.

Study II. Interval aerobic training combined with strength exercise improves metabolic markers beyond caloric restriction in Zucker rats

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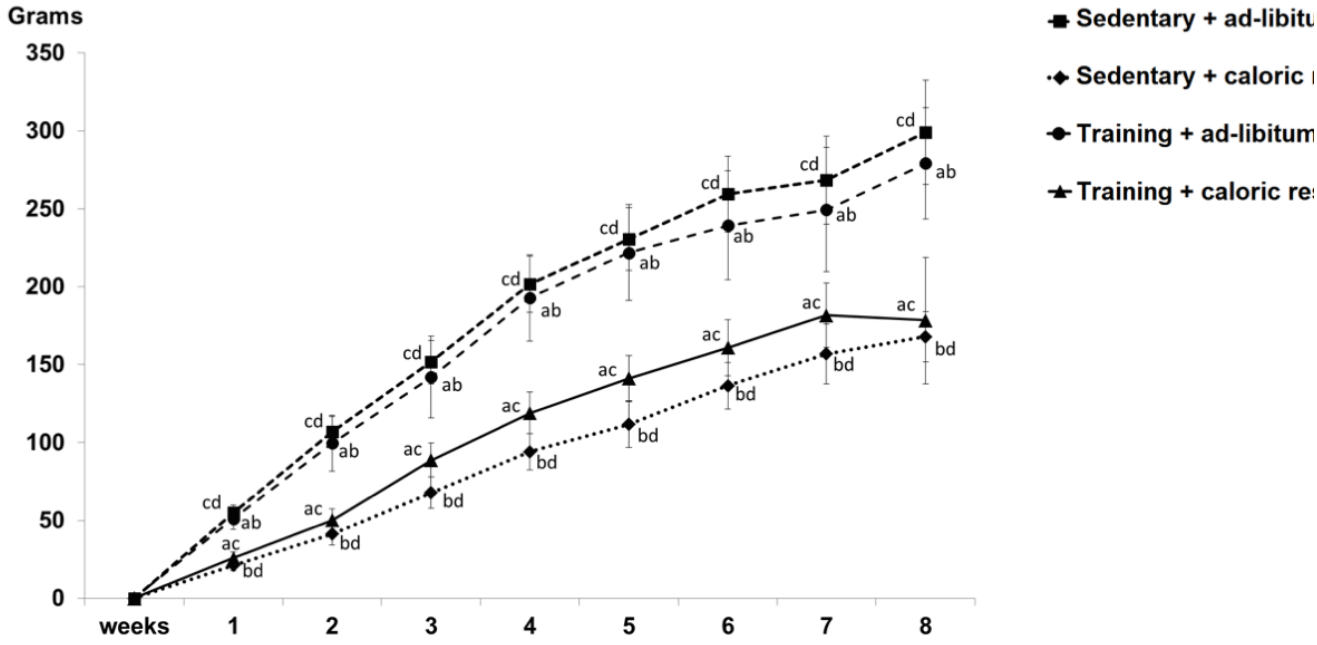
2.1. Food intake, body weight and body composition

The effects of the training protocol and CR on food intake, body composition and aerobic capacity markers are shown in Table 7. Food intake was 8% higher in the exercise groups ($P<0.001$). Final body weight was 16% lower in the CR compared to the *AL* groups ($P<0.001$). Increases in body weight along the experimental period were lower for both CR groups ($P<0.001$, Figure 4). Body fat mass was 7% lower in the CR compared to the *AL* groups ($P<0.05$) and 14% lower in the exercise compared to the sedentary groups ($P<0.001$). Body lean mass was 16% higher in the exercise compared to the sedentary groups ($P<0.001$). Total body water was 16% higher in the exercise compared to the sedentary groups ($P<0.001$).

Some exercise*CR interaction was observed on lean mass and total body water, such that sedentary+CR animals showed the lowest lean mass and total body water whereas exercise+CR animals showed the highest values (both, $P<0.01$).

Figure 5A shows the clustered adverse body composition. Exercise improved adverse body composition compared to sedentary groups, especially when it was combined with CR (overall $P<0.001$). Pairwise comparisons also showed that the exercise+*AL* group presented significantly better body composition than the sedentary+CR group.

Figure 4. Body weight gains of the experimental groups.



[a,b,c,d,e] Common superscript indicates a significant difference ($P<0.05$) between groups with the same letter. Pairwise comparisions were performed with Bonferroni's adjustment.

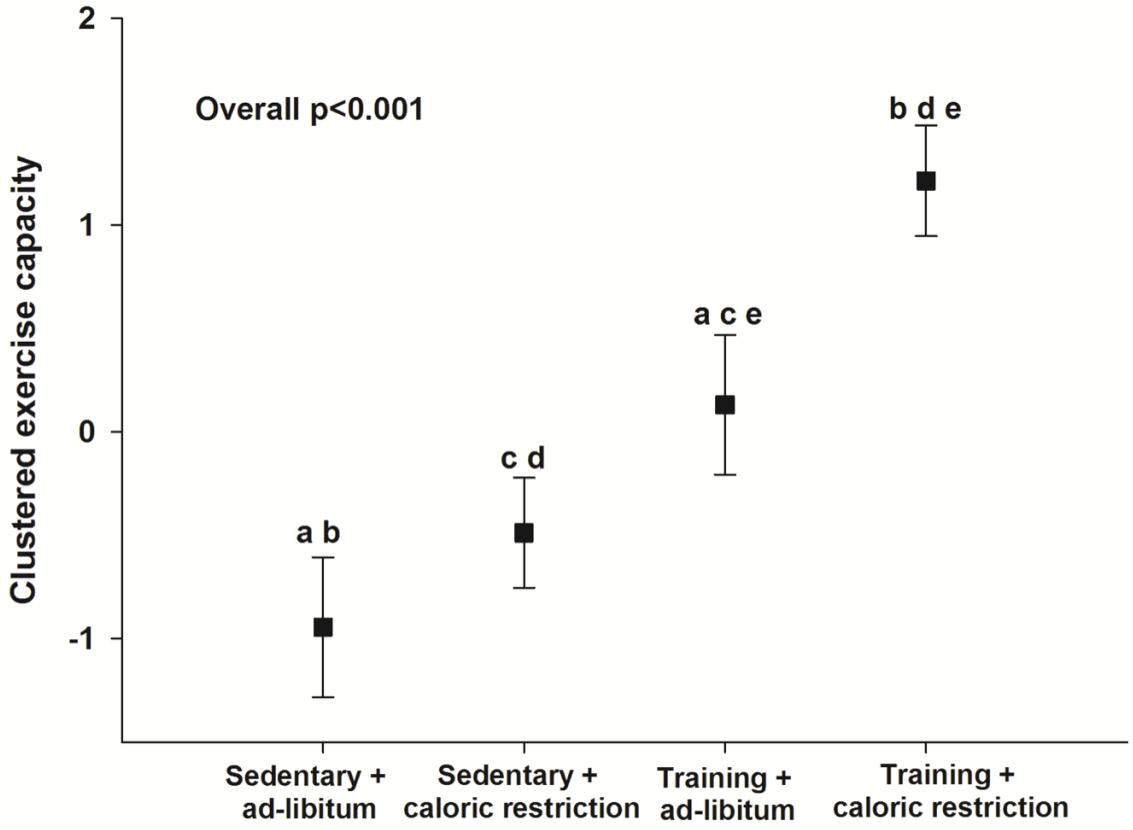
2.2. Exercise capacity and muscle development

Total running time, maximal speed and distance covered in the maximal incremental test were higher in the exercise and in the CR compared to the sedentary and *AL* groups, respectively (all, $P<0.001$). The VO_2peak was higher in the exercise compared to the sedentary groups ($P<0.001$). Quadriceps Nitrogen content and percentage of protein was 11% higher in the exercise compared to the sedentary groups ($P<0.001$) and 7% lower in the CR compared to the *AL* groups ($P<0.01$).

A significant exercise×CR interaction was found for all the aerobic capacity parameters studied, such that the highest values corresponded to the exercise+CR group (all, $P<0.01$).

Figure 6 shows the clustered exercise capacity. Exercise groups presented better clustered exercise capacity than the sedentary groups, and the exercise+CR group showed the highest exercise capacity (overall $P<0.001$).

Figure 6. Clustered exercise capacity (z-score) by experimental groups.



Dots represent mean and standard error. [a,b,c,d,e] Common superscript indicates a significant difference (P<0.05) between groups with the same letter. Pairwise comparisions were performed with Bonferroni's adjustment.

2.3. Lipid profile

The effects of the training protocol and CR on plasma lipid, glycaemic and inflammatory profile are shown in Table 8. Plasma triglycerides were 50% lower in the CR compared to the *AL* groups ($P<0.001$) without differences regarding exercise. Exercise groups reduced 24% total cholesterol, 49% LDL-C, 16% HDL-C and 12% phospholipids compared to the sedentary groups (all, $P<0.001$). Caloric restriction increased 16% HDL-C ($P<0.001$) and reduced 12% phospholipids ($P<0.05$) compared to the *AL* groups.

Some exercise×CR interactions were observed. Sedentary+CR rats had the lowest values in plasma triglycerides ($P<0.05$). The highest concentration of total cholesterol was observed in the sedentary+CR group, whereas exercise significantly reduced it by 7% ($P<0.05$). Similarly, the highest HDL-C concentration was observed in the sedentary+CR group ($P<0.01$).

Figure 5B shows the clustered adverse lipid profile. Overall, CR was more effective than exercise at improving the clustered lipid profile ($P<0.001$). Pairwise comparisons showed that the sedentary+CR group presented a lower clustered adverse lipid profile than the exercise+*AL* group ($P<0.05$).

2.4. Inflammatory markers

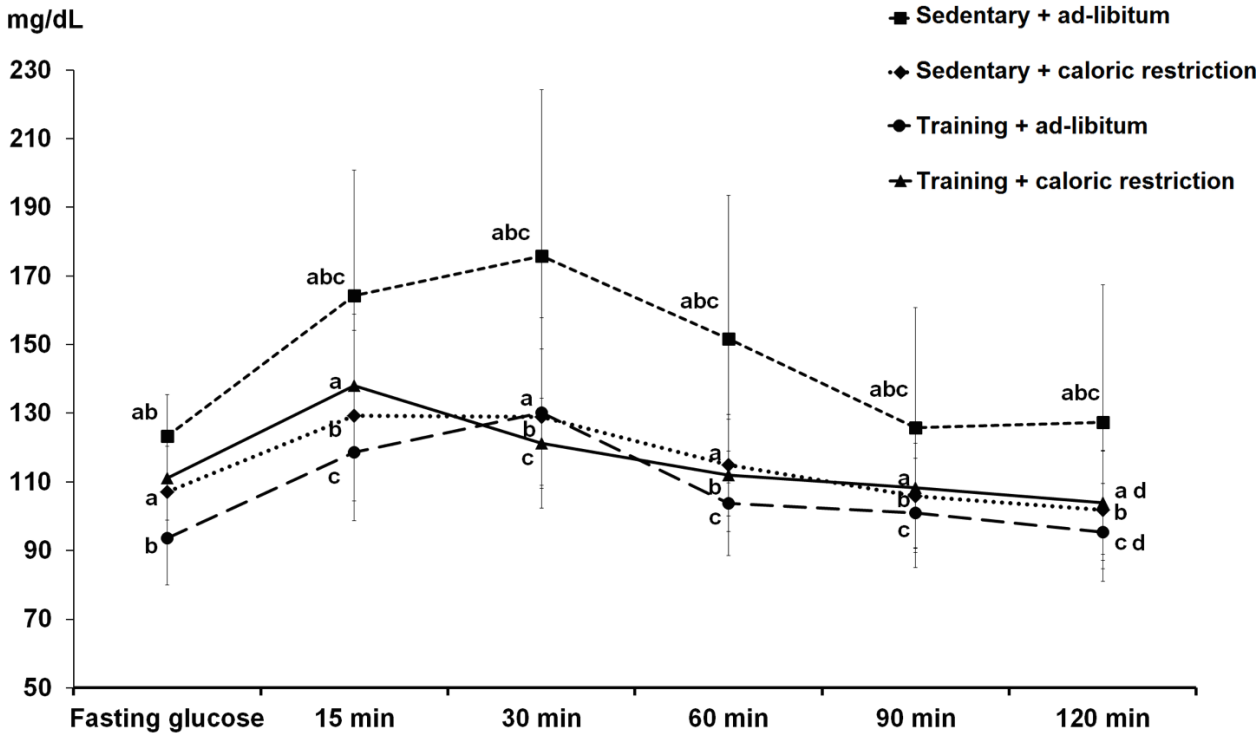
Plasma adiponectin concentrations were 20% lower in the CR compared to the *AL* groups ($P<0.001$). A significant exercise×CR interaction was found, with the lowest adiponectin levels corresponding to the exercise+CR group and the highest to the exercise+*AL* group ($P<0.05$).

2.5. Glycaemic profile

Fasting glucose was 11% lower in the exercise compared to the sedentary groups ($P<0.001$). The area under the curve after oral glucose tolerance test was 44% lower in the exercise compared to the sedentary groups and 31% lower in the CR compared to the *AL* groups (both, $P<0.001$). Plasma insulin concentrations were ~43% lower in the exercise compared to the sedentary and the CR compared to the *AL* groups (both, $P<0.001$). Homeostatic model assessment for insulin resistance was 65% lower in the exercise compared to the sedentary groups and 31% lower in the CR compared to the *AL* groups (both, $P<0.001$). Postprandial glucose after oral glucose tolerance test after 15, 30 and 60 minutes was lower in the exercise compared to the sedentary groups, and CR

groups showed lower postprandial glucose after 30 and 120 minutes compared to the *AL* groups (Figure 7). Pairwise comparisons yielded significant between-group differences, especially between the sedentary+*AL* group with the rest of interventions.

Figure 7. Fasting and postprandial glucose of the experimental groups after oral glucose tolerance test.



[a,b,c,d,e] Common superscript indicates a significant difference (P<0.05) between groups with the same letter. Pairwise comparisons were performed with Bonferroni's adjustment.

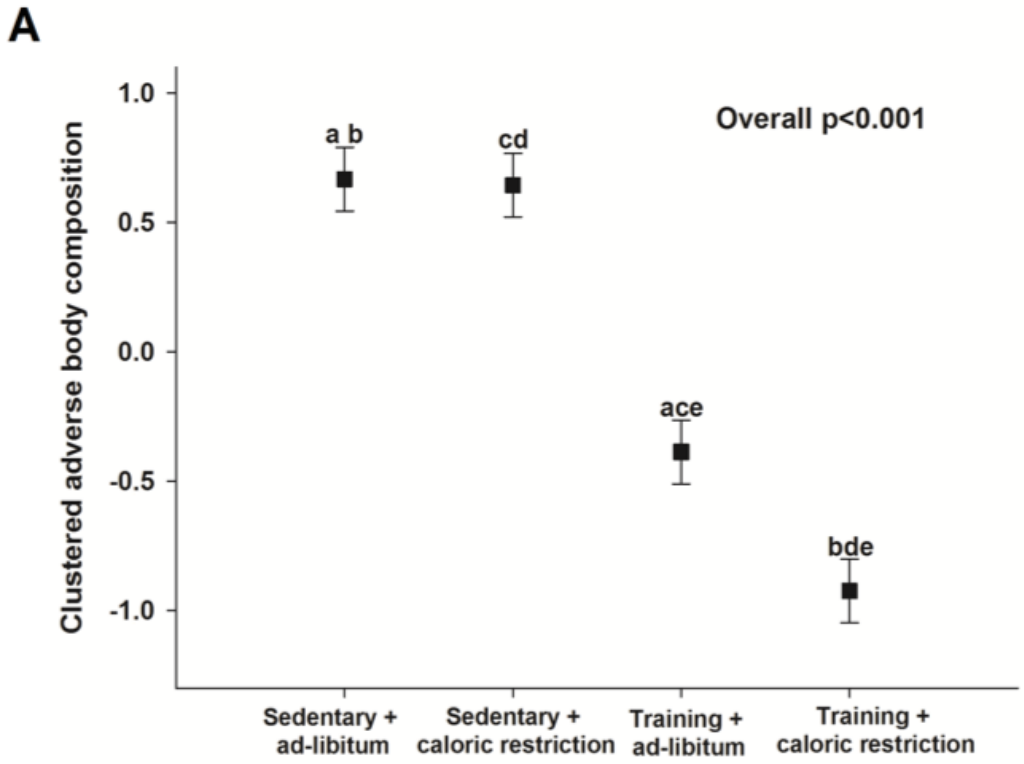
Some significant exercise×CR interactions were found on plasma glycaemic profile such as the higher reductions in fasting and postprandial glucose in the exercise+*AL* group whereas CR showed to be more effective reducing the area under the curve after oral glucose tolerance test and the HOMA-IR in the sedentary groups (both, $P<0.001$).

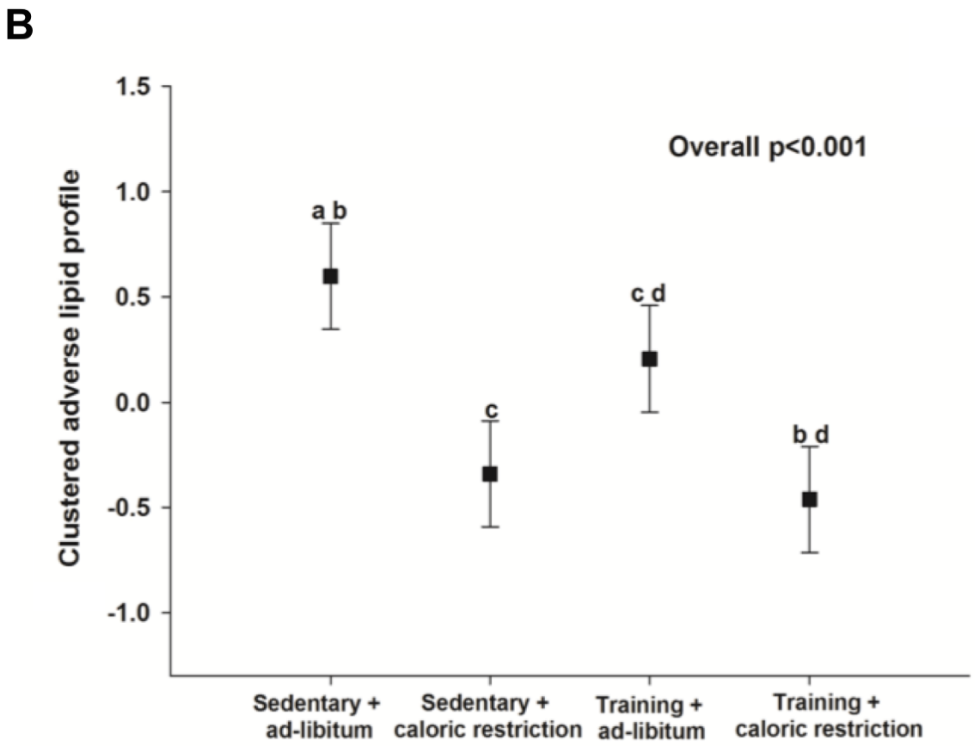
Figure 5C shows the clustered adverse glycemic profile. Sedentary+*AL* group presented greater adverse clustered glycemic profile compared to the rest of groups ($P<0.001$).

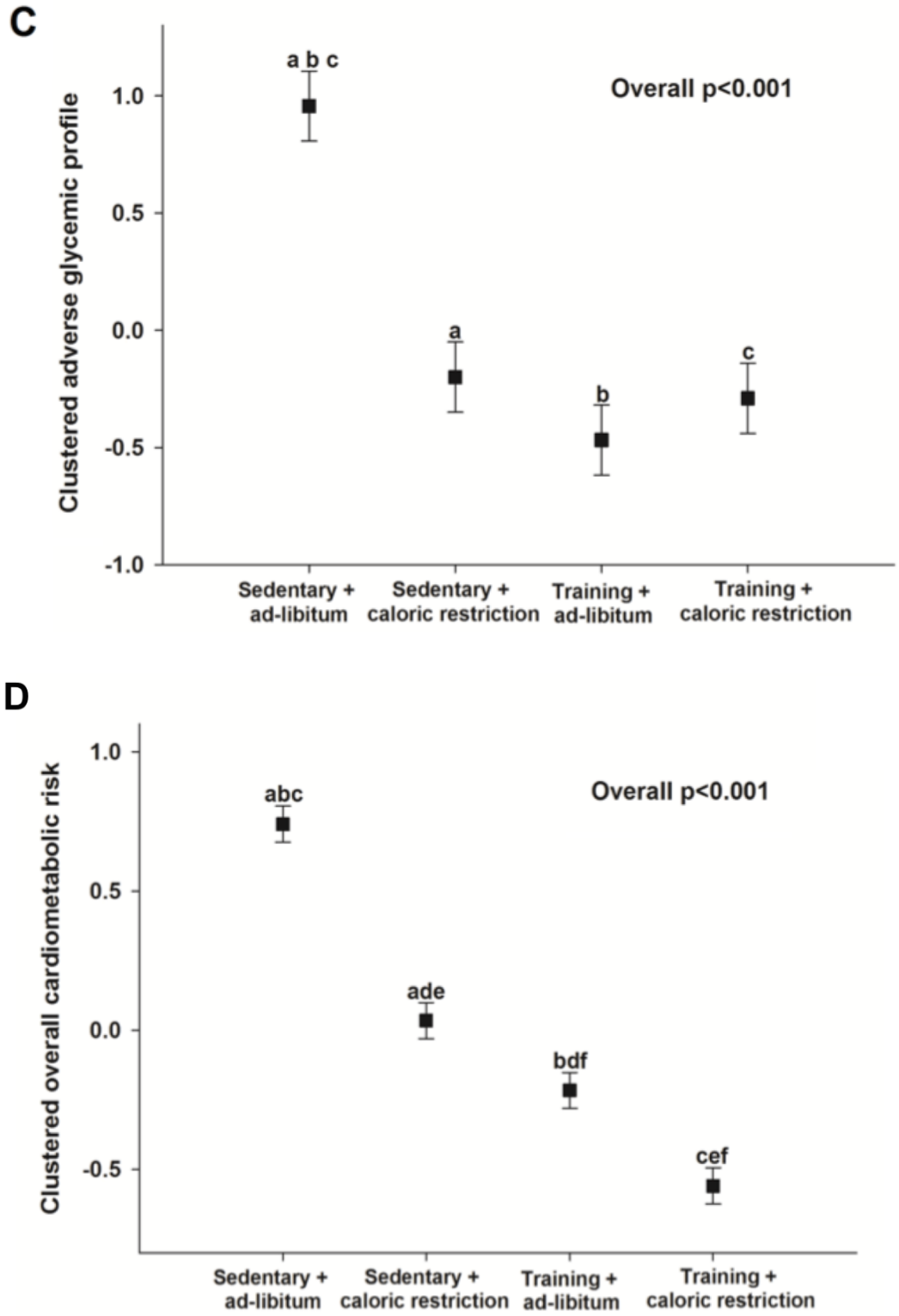
2.6. Clustered cardiometabolic risk

Figure 5D shows the clustered cardiometabolic risk. The exercise+CR group presented the lowest cardiometabolic risk (overall $P<0.001$). Pairwise comparisons showed differences between all the interventions groups, but the exercise+*AL* group presented lower clustered cardiometabolic risk than the sedentary+CR group ($P<0.05$). When further including inverse adiponectin levels into the model (Figure 8), the exercise+*AL* group showed the lowest cardiometabolic risk and the exercise+*AL* group presented lower clustered cardiometabolic risk than the sedentary+CR group ($P<0.05$).

Figure 5. Clustered (z-score) adverse body composition (5A), lipid profile (5B), glycemic profile (5C) and overall cardiometabolic risk (5D) by experimental groups.

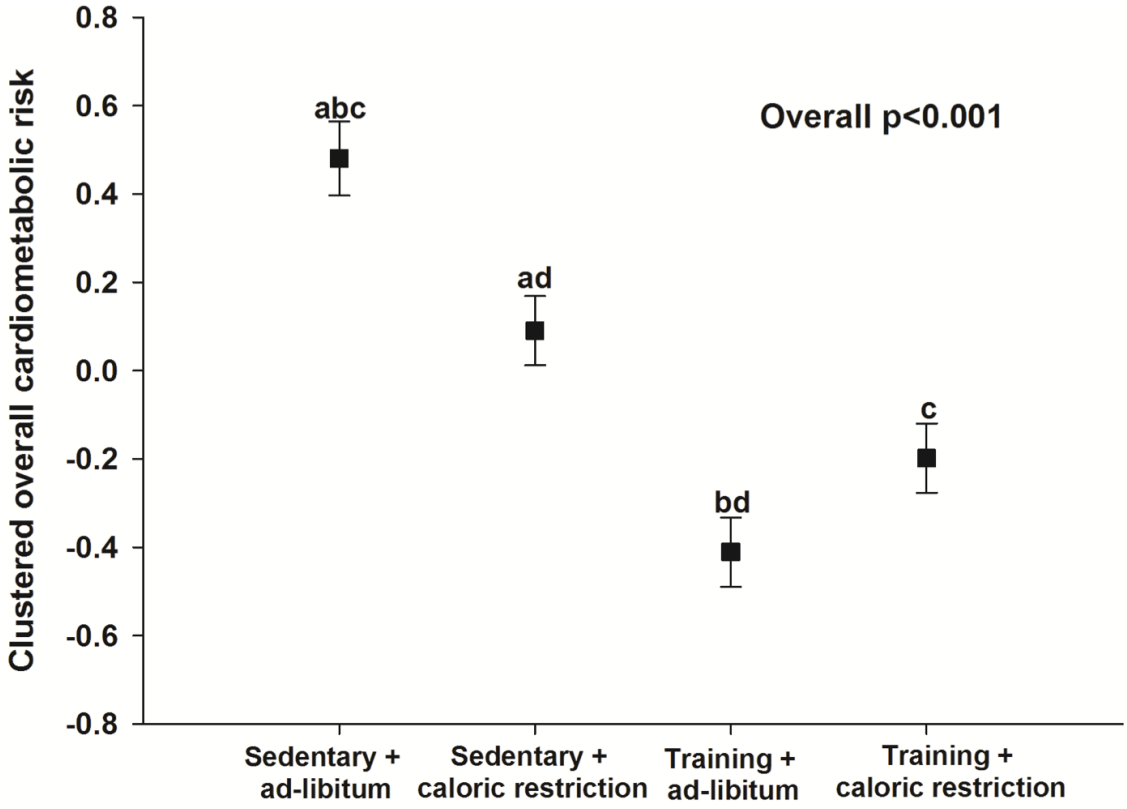






Dots represent mean and standard error. [a,b,c,d,e] Common superscript indicates a significant difference (P<0.05) between groups with the same letter. Pairwise comparisons were performed with Bonferroni's adjustment.

Figure 8. Clustered (z-score) overall cardiometabolic risk additionally including adiponectin levels by experimental groups



Dots represent mean and standard error. [a,b,c,d,e] Common superscript indicates a significant difference (P<0.05) between groups with the same letter. Pairwise comparisons were performed with Bonferroni's adjustment.

Table 7. Effects of the interval aerobic training combined with strength exercise protocol and caloric restriction (CR) on food intake, body composition and aerobic capacity markers.

	Sedentary		**Exercise**		***P***			**Effect size†**	
	Ad libitum	**CR**	***Ad libitum***	**CR**	**exercise**	**CR**	**exercise *CR**	**exercise**	**CR**
Food intake, g	25.4 (1.0)	17.7 (0.01)	27.5 (1.3)	19.2 (0.02)	<0.001	<0.001	<0.001	3.1 (1.5, 4.6)	-9.8 (-6.0, -13.6)
Body composition									
Final body weight, g	462.2 (32.9)	375.4 (13.6)	438.3 (28.2)	377.7 (41.4)	0.329	<0.001	0.238	0.4 (-0.7, 1.4)	-2.5 (-1.1, -3.9)
Carcass weight, g	218.8 (23.3)	146.9 (16.9)	193.4 (14.7)	183.5 (21.8)	0.430	<0.001	<0.001	0.3 (-0.8, 1.3)	-2.1 (-0.8, -3.5)
Fat mass, g	218.3 (13.3)	199.5 (12.1)	184.7 (21.5)	173.4 (19.6)	<0.001	0.019	0.537	-1.7 (-0.5, -3.0)	-0.9 (0.2, -2.0)
Lean mass, g	189.9 (13.8)	174.4 (8.0)	204.2 (14.8)	216.5 (17.0)	<0.001	0.747	0.008	2.1 (0.8, 3.4)	-0.1 (-1.2, 0.9)
Total body water, mL	164.4 (12.3)	155.4 (6.7)	174.7 (9.0)	188.4 (14.9)	<0.001	0.554	0.008	2.0 (0.7, 3.3)	-0.2 (-1.3, 0.8)
Aerobic capacity									
VO_2peak, mL/min/$kg^{0.75}$	9.57 (2.29)	14.50 (4.74)	17.30 (5.79)	20.21(0.27)	<0.001	0.069	<0.001	2.0 (0.7, 3.3)	1.2 (0.0, 2.3)
Running time, min	13.0 (0.5)	13.4 (1.1)	16.6 (1.5)	20.2 (1.5)	<0.001	<0.001	0.002	4.3 (2.4, 6.2)	1.7 (0.5, 3.0)
Maximal speed, cm/s	47.2 (2.0)	49.1 (4.3)	62.8 (7.7)	79.1 (6.3)	<0.001	<0.001	0.001	4.2 (2.3, 6.1)	1.8 (0.5, 3.0)
Distance, m	116.7 (15.6)	166.6 (32.1)	238.0 (58.6)	428.8 (75.9)	<0.001	<0.001	0.001	3.8 (2.0, 5.6)	2.6 (1.2, 4.0)
Muscle development									
Quadriceps, N g/100g DM	11.1 (0.96)	9.7 (0.60)	11.8 (1.56)	11.5 (0.96)	<0.001	0.002	0.062	1.2 (0.1, 2.3)	-0.8 (-1.9, 0.3)
Quadriceps protein, %	69.2 (5.04)	60.4 (3.75)	74.3 (9.68)	72.0 (5.78)	<0.001	0.002	0.062	1.3 (0.2, 2.5)	-0.9 (-2.0, 0.2)

Values shown as mean (standard deviation); N, Nitrogen; DM, dry matter; † Effects size statistics are expressed as *Cohen's d* (95% exact confidence interval); VO_2peak, maximum oxygen uptake.

Table 8. Effects of interval aerobic training combined with strength exercise protocol and caloric restriction (CR) on plasma lipid, glycaemic and inflammatory profile.

	Sedentary		**Exercise**		***P***			**Effect size†**	
Lipid profile	***Ad libitum***	**CR**	***Ad libitum***	**CR**	**exercise**	**CR**	**exercise *CR**	**exercise**	**CR**
Triglycerides, mg/dL	455.6 (27.7)	156.3 (27.7)	430.2 (28.6)	282.2 (28.6)	0.080	<0.001	0.010	1.8 (0.5, 3.0)	-7.9 (-4.8, -11.1)
Total cholesterol, mg/dL	223.6 (8.6)	252.7 (8.6)	188.2 (8.9)	174.5 (8.9)	<0.001	0.385	0.017	-6.5 (-3.9, -9.1)	0.9 (0.2, 2.0)
LDL cholesterol, mg/dL	57.7 (5.1)	66.2 (5.1)	29.5 (5.3)	34.0 (5.3)	<0.001	0.219	0.704	-5.8 (-3.4, -8.2)	1.3 (0.1, 2.4)
HDL cholesterol, mg/dL	71.3 (3.0)	94.2 (3.0)	69.6 (3.0)	69.7 (3.0)	<0.001	<0.001	<0.001	-4.4 (-2.4, -6.3)	3.8 (2.1, 5.6)
Phospholipids, mg/dL	365.1 (9.5)	345.3 (9.5)	325.5 (9.7)	300.9 (9.7)	<0.001	0.025	0.805	-4.3 (-2.4, -6.3)	-2.3 (-1.0, -3.7)
Glycaemic profile									
Fasting glucose, mg/dL	123.4 (12.1)	107.1 (13.2)	93.7 (13.7)	111.1(12.1)	<0.001	0.854	<0.001	-1.0 (0.1, -2.1)	0.0 (-1.1, 1.0)
Area under the curve, AU	6190 (1912)	2081 (665)	2251 (750)	2350 (940)	<0.001	<0.001	<0.001	-1.7 (-0.5, -2.9)	-1.8 (-0.6, -3.1)
Insulin, pg/mL	225.6 (39.8)	130.6 (24.4)	126.8 (25.6)	73.1 (18.4)	<0.001	<0.001	0.048	-2.8 (-1.3, -4.3)	-2.7 (-1.2, -4.1)
HOMA-IR	6.11 (1.88)	2.59 (0.74)	1.55 (0.58)	1.48 (0.44)	<0.001	<0.001	<0.001	-2.8 (-1.4, -4.3)	-1.9 (-0.6, -3.1)
Inflammatory markers									
IL-1, pg/mL	123.7 (21.6)	79.3 (33.0)	75.4 (22.2)	43.7 (33.3)	0.194	0.201	0.855	-1.5 (-0.3, -2.7)	-1.4 (-0.2, -2.5)
IL-10, pg/mL	173.6 (32.1)	152.3 (41.0)	130.7 (33.0)	73.1 (67.0)	0.365	0.192	0.718	-1.7 (-0.5, -3.0)	-1.1 (-0.0, -2.3)
TNF-α, pg/mL	0.86 (0.20)	1.06 (0.45)	0.98 (0.24)	0.39 (0.30)	0.538	0.436	0.228	-0.9 (0.2, -2.0)	-0.6 (0.5, -1.7)
Adiponectin, ng/mL	160.0 (26.8)	146.4 (1.6)	186.3 (33.8)	126.6 (12.9)	0.708	<0.001	0.012	0.2 (-0.9, 1.2)	-1.7 (-0.4, -2.9)

Values shown as mean (standard deviation); † Effects size statistics are expressed as *Cohen's d* (95% exact confidence interval). LDL, low density lipoprotein; HDL; high density lipoprotein; AU, arbitrary units; HOMA-IR, homeostatic model assessment for insulin resistance; IL, interleukin; TNF-α, tumour necrosis factor alpha; CR, caloric restriction.

Stage 2. Interval aerobic exercise combined with strength training on climacteric women.

The three next studies are part of the FLAMENCO Project sharing the following anthropometric, clinic and sociodemographic information. Afterwards, the results of each individual study are presented below this information.

The baseline characteristics of the study participants by group (counselling or exercise) are shown in Table 9. Women, who were 52.8 ±4.5 years old had a mean BMI of 27.6 Kg/m^2 in the counselling group and 27.5 Kg/m^2 in the exercise group. Furthermore, the participants were involved in moderate-vigorous physical activity for around 180 minutes/week and they showed a moderate adherence to the Mediterranean Diet[82]. Counselling and exercise groups scored a mean of 15.0 and 16.6 points, respectively, in the Blatt-Kupperman index assessed at baseline, being both groups mildly affected by menopause[79]. Less than 30% of the women had regular menstruation at the time of the first evaluation. Roughly, almost 70% of the women were married and worked full or part-time, almost 70% had finished secondary school or professional training, and 30% had University studies. No differences between the counselling and exercise groups were found in any of these variables studied (all, $P>0.05$).

Table 9. Baseline anthropometric and sociodemographic characteristics of the study participants (n=150) of the Flamenco Project.

	Counselling Group	**Exercise Group**
	mean (SD) (n=75)	**mean (SD) (n=75)**
Age, years	52.7 (4.51)	52.8 (4.48)
Height, cm	159 (5.78)	159 (6.04)
Weight, kg	70.2 (12.0)	69.7 (12.8)
Body Mass Index, kg/m2	27.6 (4.20)	27.5 (4.86)
Lean Mass index, kg/m2	14.8 (1.57)	14.7 (1.80)
Fat Mass Index, kg/m2	11.5 (2.90)	11.5 (3.13)
Moderate-vigorous physical activity, min/week	181 (145)	179 (157)
Mediterranean diet score	30.7 (4.78)	31.1 (3.85)
Women meeting PA recommendations*, %	54.5	45.5
Kupperman global score (0-45)	15.0 (10.2)	16.7 (10.8)
Sociodemographic characteristics	**n (%)**	**n (%)**
Regular menstruation (yes)	22 (29.3)	19 (25.3)
Marital status		
Married or with partner	55 (73.3)	50 (67.6)
Single	6 (8.0)	11 (14.9)
Divorced/widow	14 (18.6)	13 (17.65)
Educational level		
No studies	2 (2.7)	2 (2.7)
Primary school	21 (28.0)	18 (24.3)
Secondary school /professional training	28 (37.3)	28 (37.3)
University degree	24 (32.0)	26 (35.7)
Occupational status		
Working full-time	33 (44.2)	21 (28.4)
Working part-time	9 (12.3)	16 (21.6)
Housewife	31 (43.5)	37 (50.0)

SD, Standard Deviation; PA, Physical activity; *Minimum recommendations for this population are 150min/week of moderate to vigorous physical activity in bouts of at least 10 minutes.

Study III. Body composition changes in perimenopausal women following a concurrent exercise intervention: The FLAMENCO Project Randomised Controlled Trial

3.1. Body composition

Table 10 shows the baseline values for body composition variables and for pharmaceutical costs of the participants. No differences between the counselling and exercise groups were found in any of the variables studied (all $P>0.05$).

3.1.1. Intention-to-treat analyses

Table 11 shows the intention-to-treat analyses of body composition changes between pre and post intervention for counselling and exercise groups. Compared to the counselling group, the exercise group lost -0.75 (95% CI: -1.29 to -0.22; $P<0.01$) kg/m^2 of BMI. Gynoid fat mass (between-group difference: -115g; 95% CI -209 to -3.86; $P<0.05$) and android fat mass (between-group difference: -92.9g; 95% CI -164 to -26.9; $P<0.01$) decreased significantly in the exercise group. Compared to the exercise group, the counselling group lost -3.98 (95% CI: 0.93 to 7.81; $P<0.05$) of BMC of pelvis. No differences between groups were observed for lean mas, fat mass, visceral adipose tissue, total BMD, total BMC, pelvis and lumbar spine BMD.

3.1.2. Per-protocol analyses

The per-protocol analysis (Table 12) showed similar results in BMI, android fat mass, and BMC of pelvis, but no differences were found in gynoid fat mass. Total BMC 23.5 ml/dL less in the exercise group (95% CI: 0.38 to 69.0; $P<0.05$).

Compared to the exercise group, the counselling group expenditures in pharmaceutical costs increased 15.7€ per month (95% CI: -27.5 to -2.77; $P<0.05$).

3.2. Pharmaceutical costs

An exploratory analysis was performed to study the association between body composition improvements seen in the intention-to-treat analysis, and pharmaceutical costs changes (Table13). The improvements found in BMI and BMC of pelvis after the intervention showed no association with reduced pharmaceutical expenditures [(95% CI:

-1.29 to 5.39; *P*>0.05) and (95% CI: -0.74 to 0.27; *P*>0.05), respectively]. The improvement found in android fat mass after the exercise programme reduced the pharmaceutical expenditures (95% CI: 0.01 to 0.06; *P*<0.05) and the improvement found in gynoid fat mass after the exercise programme was also associated with lower pharmaceutical costs (95% CI: 0.01 to 0.04; *P*<0.01).

Table 10: Baseline values of body composition of the climacteric women (n=150).

	Counselling Group	**Exercise Group**
	mean (SD) (n=75)	**mean (SD) (n=75)**
Baseline body composition		
Weight, kg	70.1 (12.0)	69.7 (12.8)
Body Mass Index, kg/m^2	27.6 (4.2)	27.5 (4.9)
Lean mass, kg	37.7 (4.8)	37.4 (4.9)
Fat mass, kg	29.4 (7.6)	29.2 (7.9)
Fat mass percentage, %	42.0 (4.9)	41.9 (4.8)
Visceral adipose tissue, kg	0.71 (0.3)	0.66 (0.3)
Gynoid fat mass, kg	5.1 (6.3)	5.2 (1.3)
Android fat mass, kg	2.5 (0.9)	2.5 (0.9)
Total bone mineral density, g/cm2	1.14 (0.1)	1.15 (0.1)
Total bone mineral content, g	2117.2 (286)	2157.2 (294)
Bone mineral content of pelvis, g	185.8 (43.7)	191.3 (43.6)
Bone mineral density of pelvis, g/cm^2	1.12 (0.1)	1.12 (0.1)
Bone mineral density of lumbar spine, g/cm^2	0.88 (0.1)	0.89 (0.1)
Costs of medication		
Monthly pharmaceutical cost, €	20.1 (38.0)	27.7 (54.9)

SD, Standard Deviation.

Table 11. Intention-to-treat analyses showing the influence of a 4-month interval aerobic training combined with strength exercise program compared to counselling on body composition in climacteric women (n=150).

Body composition	**Counselling (n=75) Differences from baseline to week 16 (SD)**	**Exercise (n=75) Differences from baseline to week 16 (SD)**	**Between group-difference (95% CI)**	***P***
Weight*, kg	-0.07 (3.48)	-1.52 (6.30)	-1.45 (-3.32, 0.39)	0.121
Body Mass Index*, kg/m^2	0.00 (1.34)	-0.75 (1.96)	-0.75 (-1.29, -0.22)	0.006
Lean mass*, g	471 (1280)	314 (1416)	-157 (-592, 284)	0.489
Fat mass*, g	-544 (1081)	-903 (2159)	-359 (-903, 175)	0.184
Fat mass percentage*, %	-0.72 (1.24)	-0.95 (2.20)	-0.23 (-0.80, 0.32)	0.396
Visceral adipose tissue*, g	-21.3 (67.0)	-30.5 (88.3)	-9.2 (-37.1, 12.1)	0.315
Gynoid fat mass*, g	-98.3 (221)	-213 (414)	-115 (-209, -3.86)	0.042
Android fat mass*, g	-46.1 (152)	-139 (268)	-92.9 (-164, -26.9)	0.007
Total bone mineral density[a], g/cm^2	-0.01 (0.02)	0.00 (0.02)	0.01 (-0.00, 0.02)	0.114
Total bone mineral content[a], g	-23.6 (104)	-8.65 (34.5)	15.0 (-6.20, 43.69)	0.140
Bone mineral content of pelvis[a], g	-2.85 (10.2)	1.13 (11.5)	3.98 (0.93, 7.81)	0.013
Bone mineral density of pelvis[a], g/cm^2	-0.03 (0.03)	0.00 (0.03)	0.03 (-0.00, 0.02)	0.171
Bone mineral density of lumbar spine[a], g/cm^2	-0.01 (0.07)	0.00 (0,08)	0.01 (-0.01, 0.03)	0.319
Pharmaceutical cost per woman*, €/month	12.3 (34.4)	2.95 (35.8)	-9.35 (-19.4, 3.22)	0.160

Mean results show the differences between post-pre intervention results for each variable. ITT, Intention-to-treat analyses; SD, Standard Deviation. *Model adjusted for baseline value of the variable. [a]Model adjusted for baseline value of the variable, age, height and fat and lean mass.

Table 12. Per-protocol analyses showing the influence of a 4-month interval aerobic training combined with strength exercise program compared to counselling on body composition in climacteric women (n=114).

Body composition	**Counselling (n=55) Differences from baseline to week 16 (SD)**	**Exercise (n=59) Differences from baseline to week 16 (SD)**	**Between-group difference (95%CI)**	***P***
Weight*, kg	-0.01 (3.49)	-1.69 (6.65)	-1.68 (-3.18, 0.23)	0.090
Body Mass Index*, kg/m^2	0.03 (1.61)	-0.91 (2.16)	-0.94 (-1.46, -0.12)	0.022
Lean mass*, g	655 (1474)	344 (1478)	-311 (-841, 288)	0.334
Fat mass*, g	-758 (1213)	-1063 (2257)	-305 (-984, 426)	0.435
Fat mass percentage*, %	-1.00 (1.37)	-1.12 (2.26)	-0.02 (-0.82, 0.60)	0.743
Visceral adipose tissue*, g	-29.7 (77.6)	-32.6 (92.0)	-2.9 (-37.8, 27.6)	0.759
Gynoid fat mass*, g	-136 (250)	-242 (435)	-106 (-213, 55.1)	0.245
Android fat mass*, g	-64.2 (176)	-162 (283)	-97.8 (-197, -18.5)	0.018
Total bone mineral density, g/cm2	-0.01 (0.02)	0.00 (0.02)	0.01 (0.00, 0.01)	0.110
Total bone mineral content[a], g	-32.9 (121)	-9.38 (34.9)	23.5 (0.38, 69.0)	0.048
Bone mineral content of pelvis[a], g	-3.97 (11.9)	1.51 (12.2)	5.48 (0.67, 0.97)	0.025
Bone mineral density of pelvis[a], g/cm^2	0.00 (0.03)	0.01 (0.03)	0.01 (-0.01, 0.01)	0.734
Bone mineral density of lumbar spine[a], g/cm^2	-0.01 (0.08)	0.00 (0.08)	0.01 (-0.01, 0.05)	0.200
Pharmaceutical cost per woman*, €/month	13.6 (34.5)	-2.01 (28.3)	-15.7 (-27.5, -2.77)	0.017

Mean results show the differences between post-pre intervention results for each variable. SD, Standard Deviation. *Model adjusted for baseline value of the variable. [a]Model adjusted for baseline value of the variable, age, height and fat and lean mass.

[¶] Only women with available data and participants in the exercise group who attended ≥75% of the exercise sessions were included.

Table 13: Association between the change on body composition and changes in pharmaceutical expenses.

	Unstandardized Coefficients	Standardized Coefficients		Confidence interval 95% (B)	
	B	**Beta**	***P***	**Lower**	**Upper**
Body Mass Index, kg/m2	2.05	0.10	0.228	-1.29	5.39
Android fat mass, g	0.03	0.21	0.011	0.01	0.06
Gynoid fat mass, g	0.02	0.23	0.006	0.01	0.04
Bone mineral content of pelvis, g	-0.24	0.08	0.360	-0.74	0.27

Study IV. Effects of concurrent exercise on cardiometabolic status during perimenopause. The FLAMENCO Project

4.1. Cardiometabolic profile

Table 14 shows the baseline values for cardiometabolic variables of the participants. No differences between the counselling and exercise groups were found in any of the variables studied (all P>0.05).

4.1.1. Per-protocol analyses

Table 15 shows the per-protocol analyses of cardiometabolic markers changes between pre and post intervention for counselling and exercise groups. For this specific study, per-protocol analysis was considered as the main one, as a high number of data had to be imputed and may be due to chance. In per-protocol analysis HDL-C and triglycerides increased in both groups. Compared to the counselling group, the exercise group lost -9.58 mg/dL of LDL-C when adjusting for the baseline values, and -10.2 mg/dL when adjusting for the baseline values and Mediterranean Diet Score (95% CI: -19.4 to -0.96; *P*<0.05). Borderline significant but clinically meaningful differences[84] were found between groups in plasma glucose when adjusting for the baseline values (95% CI: -0.39 to 6.13; *P*=0.083), with a greater decrease 2.9 ml/dL in the counselling group, and similar results when adjusting for the baseline values and Mediterranean Diet Score (95% CI: -0.50 to 6.29; *P*=0.093). Total cholesterol also showed borderline significant changes, with a greater decrease of -9.6 ml/dL in the exercise group when adjusting for the baseline value and Mediterranean Diet Score (95% CI: -20.0 to 0.74; *P*=0.068). Finally, Diastolic blood pressure also showed borderline significant changes, with a greater decrease of -2.2 mmHg in the exercise group when adjusting for the baseline value (95% CI: -4.58 to 0.25; *P*=0.078), and similar results when adjusting for the baseline value and Mediterranean Diet Score (-2.1 mmHg; 95% CI: -4.50 to 0.33; *P*=0.090). No differences between groups were observed for HDL-C, triglycerides, or CRP.

4.1.2. Intention-to-treat analyses

Table 16 shows the intention-to-treat analyses of cardiometabolic markers changes between pre and post intervention for counselling and exercise groups. Intention to treat

results showed a similar tendency, the counselling group reduced plasma glucose -3.8 ml/dL (95% CI: 0.97, 6.54; *P*<0.01) compared with the exercise group, the exercise group increased the HDL-C by 2.9 ml/dL (95% CI: -0.01, 5.72; *P*=0.051) compared to the counselling, and a difference of 0.7 ml/dL of CRP, decreasing less in the exercise group (95% CI: 0.08 to 1.30; *P*<0.05), all adjusted for the baseline values. The model adjusted for the baseline values and Mediterranean Diet Score showed similar results.

Table 14. Baseline values of body composition, cardiometabolic profile, and sociodemographic characteristics for counselling and exercise groups (n=150).

	Counselling Group	**Exercise Group**
	mean (SD)	**mean (SD)**
	(n=75)	**(n=75)**
Cardiometabolic profile		
Glucose, mg/dL	87.3 (9.2)	90.2 (13.4)
Total cholesterol, mg/dL	222.9 (21.9)	224.8 (35.2)
LDL cholesterol, mg/dL	142. 1(17.6)	143.1 (31.2)
HDL cholesterol, mg/dL	60.7 (15.0)	60.1 (11.2)
Triglycerides, mg/dL	107.2 (51.4)	106.4 (48.7)
C-reactive protein, mg/dL	3.80 (4.3)	3.53 (4.1)
Systolic blood pressure, mmHg	121 (15.9)	122 (14.3)
Diastolic blood pressure, mmHg	75.6 (9.7)	76.3 (8.7)
Resting heart rate, beats/min	73.7 (9.3)	78.2 (11.5)

SD, Standard Deviation; LDL, low density lipoprotein; HDL, high density lipoprotein.

Table 15. Per-protocol analyses showing the influence of a 4-month interval aerobic training combined with strength exercise program compared to counselling on plasma glucose, lipid profile, C-reactive protein, blood pressure and heart rate after in climacteric women (n=112) ¶.

			Unadjusted difference				Model adjusted for baseline values of each variable				Model adjusted for baseline values and MDS			
	Changes in counselling group (n=20)	Changes in exercise group (n=41)	B	CI (95%) for B	β	*P*	B	CI (95%) for B	β	*P*	B	CI (95%) for B	β	*P*
Glucose, ml/dL	-6.19 (6.15)	-4.07 (6.88)	2.123	(-1.43, 5.68)	0.151	0.237	2.872	(-0.39, 6.13)	0.205	0.083	2.895	(-0.50, 6.29)	0.202	0.0
Total cholesterol, ml/dL	-11.5 (18.0)	-21.8 (25.2)	-10.28	(-22.6, 2.07)	-0.208	0.101	-7.921	(-18.1, 2.23)	-0.161	0.124	-9.644	(-20.0, 0.74)	-0.192	0.0
LDL cholesterol, ml/dL	-14.5 (18.7)	-23.9 (19.0)	-9.426	(-20.2, 1.38)	-0.229	0.086	-9.584	(-18.7, -0.42)	-0.233	0.041	-10.21	(-19.4, -0.96)	-0.247	0.0
HDL cholesterol, ml/dL	0.64 (7.39)	0.90 (7.37)	0.250	(-3.85, 4.35)	0.016	0.903	0.772	(-3.20, 4.74)	0.049	0.699	0.773	(-3.29, 4.84)	0.049	0.7
Triglycerides, ml/dL	7.74 (45.5)	10.3 (49.9)	2.533	(-24.7, 29.7)	0.025	0.853	2.823	(-23.4, 29.1)	0.028	0.830	2.672	(-24.2, 29.6)	0.026	0.8
C-reactive protein, ml/dL	-0.81 (3.42)	-0.74 (3.31)	0.073	(-1.74, 1.89)	0.010	0.936	0.362	(-0.74, 1.47)	0.051	0.515	0.371	(-0.78, 1.52)	0.052	0.5
	n=54	**n=58**												
Systolic blood pressure, mmHg	-7.63 (12.7)	-6.93 (12.1)	0.699	(-3.96, 5.36)	0.028	0.767	1.083	(-3.16, 5.33)	0.044	0.614	1.736	(-2.52, 6.00)	0.071	0.4
Diastolic blood pressure, mmHg	-3.44 (7.50)	-5.66 (5.58)	-2.211	(-4.68, 0.25)	-0.167	0.078	-2.167	(-4.58, 0.25)	-0.164	0.078	-2.082	(-4.50, 0.33)	-0.157	0.0
Resting heart rate, beats/min	1.67 (6.75)	-0.38 (9.53)	-2.046	(-5.16, 1.07)	-0.123	0.196	-0.612	(-3.63, 2.40)	-0.037	0.688	-0.298	(-3.29, 2.70)	-0.018	0.8

Mean results show the differences between post-pre intervention results for each variable with negative values as a reduction in the post evaluation compared to pre-evaluation; (standard deviation) unless otherw indicated; β, standardized beta; MDS, Mediterranean Diet Score; LDL, Low-density-lipoprotein; HDL, High-density-lipoprotein; CI, Confidence interv

¶ Only women with available data and participants in the exercise group who attended ≥75% of the exercise sessions were included.

Table 16. Intention-to-treat analyses showing the influence of a 4-month interval aerobic training combined with strength exercise program compared to counselling on plasma glucose, lipid profile, C-reactive protein, blood pressure and heart rate in climacteric women (n=150).

			Unadjusted Model				Model adjusted for baseline values				Model adjusted for baseline values and MDS			
	Change in counselling group (n=75)	**Change in exercise group (n=75)**	**B**	**CI (95%) for B**	**β**	***P***	**B**	**CI (95%) for B**	**β**	***P***	**B**	**CI (95%) for B**	**β**	***P***
Glucose, mg/dL	-7.36 (8.99)	-4.10 (8.56)	3.261	(0.43, 6.09)	0.184	0.024	3.752	(0.97, 6.54)	0.212	0.009	3.793	(1.00, 6.59)	0.214	0.008
Total cholesterol, mg/dL	-17.4 (18.9)	-21.7 (28.6)	-4.229	(-12.1, 3.60)	-0.087	0.287	-2.944	(-9.40, 3.51)	-0.061	0.369	-3.281	(-9.64, 3.08)	-0.068	0.310
LDL cholesterol, mg/dL	-20.4 (15.4)	-22.6 (20.9)	-2.218	(-8.13, 3.70)	-0.061	0.460	-1.738	(-6.52, 3.04)	-0.048	0.473	-2.054	(-6.71, 2.60)	-0.056	0.385
HDL cholesterol, mg/dL	-0.80 (10.2)	2.10 (9.04)	2.898	(-0.21, 6.01)	0.150	0.067	2.854	(-0.01, 5.72)	0.148	0.051	2.750	(-0.10, 5.60)	0.142	0.058
Tryglicerides, mg/dL	16.9 (63.7)	3.66 (78.6)	-13.21	(-36.3, 9.87)	-0.093	0.260	-13.33	(-36.0, 9.32)	-0.093	0.247	-13.08	(-35.7, 9.72)	-0.091	0.260
C-reactive protein, mg/dL	-1.33 (3.52)	-0.56 (3.35)	0.768	(-0.34, 1.88)	0.112	0.173	0.689	(0.08, 1.30)	0.100	0.027	0.692	(0.08, 1.31)	0.101	0.027
Systolic blood pressure, mmHg	-8.12 (12.8)	-6.34 (12.3)	1.783	(-2.28, 5.84)	0.071	0.387	1.934	(-1.53, 5.40)	0.077	0.272	1.957	(-1.52, 5.44)	0.078	0.268
Diastolic blood pressure, mmHg	-3.57 (7.36)	-5.09 (6.11)	-1.513	(-3.70, 0.67)	0.112	0.173	-1.402	(-3.47, 0.67)	-0.104	0.183	-1.334	(-3.40, 0.73)	-0.099	0.204
Resting heart rate, beats/min	1.56 (6.78)	-0.53 (9.72)	-2.088	(-4.79, 0.62)	-0.124	0.129	-1.091	(-3.70, 1.52)	-0.065	0.410	-1.092	(-3.72, 1.53)	-0.065	0.412

Mean results show the differences between post-pre intervention results for each variable with negative values as a reduction in the post evaluation compared to pre-evaluation; (standard deviation) unless otherwise indicated; β, standardized beta; MDS, Mediterranean Diet Score; LDL, Low-density-lipoprotein; HDL, High-density-lipoprotein; CI, Confidence interval.

Study V. Influence of participating in a group exercise program on dietary behavior. The after-training beer phenomena. Findings from the FLAMENCO Project

5.*1. Dietary habits*

Table 17 shows the baseline values for dietary habits and Mediterranean Diet Score of the participants. No differences between the counselling and exercise groups were found in any of the variables studied (all $P>0.05$).

5.1.1. Intention-to-treat analyses

The changes of dietary patterns between pre and post intervention for counselling and exercise groups are shown in Table 18. Results after unadjusted model showed an increased fish intake of 1.20 (95% CI: 0.23, 2.17; $P<0.05$) servings/week in the exercise compared to the counselling group, and an increased beer intake of 1.46 (95% CI: 0.54, 2.39; $P<0.01$) servings/week in the exercise compared to the counselling group.

After adjusting the model for baseline values, results showed that fish intake was reduced in the counselling and increased in the exercise group, with a difference between groups of 1.11 (95% CI: 0.34, 1.88; $P<0.01$) servings/week. The exercise group reduced olive oil intake in -0.34 (95% CI: -0.64, -0.05; $P<0.05$) servings/day when compared to counselling group. Finally, the counselling group reduced beer intake and the exercise group increased it with a difference between groups of 1.06 (95% CI: 0.37, 1.75; $P<0.01$) servings/week.

5.1.1. Per-protocol analyses

Further analysis made with per protocol data (only those who had been assessed pre and post intervention and attended at least 75% of the sessions) showed similar results (Table 19).

Table 17. Baseline values of dietary habits and Mediterranean Diet Score in climacteric women (n=150).

	Counselling Group	**Exercise Group**
	mean (SD) (n=75)	**mean (SD) (n=75)**
Food groups		
Cereals, svgs/day	2.76 (1.22)	3.06 (1.13)
Non-refined cereals, svgs/day	0.77 (0.82)	0.83 (0.80)
Potatoes, svgs/wk	2.13 (1.48)	2.48 (2.16)
Fruit, svgs/day	2.10 (1.30)	2.52 (1.58)
Vegetables, svgs/day	4.56 (3.28)	4.76 (2.58)
Pulses, svgs/wk	2.62 (1.76)	2.54 (1.10)
Fish/shellfish, svgs/wk	5.98 (3.28)	5.84 (2.52)
Red and cured meats, svgs/wk	7.38 (4.47)	7.85 (4.43)
Poultry, svgs/wk	5.32 (2.88)	5.74 (3.87)
Dairy products, svgs/day	3.00 (1.51)	2.65 (1.17)
Whole-fat dairy products, svgs/day	0.86 (0.83)	0.84 (1.00)
Olive oil, svg/day	2.20 (1.02)	2.08 (1.10)
Red wine, svgs/wk	0.75 (0.43)	0.63 (0.49)
Beer, svgs/wk	2.49 (3.35)	1.90 (2.13)
Mediterranean diet score (0-55)	30.7 (4.78)	31.1 (3.85)

SD, Standard Deviation.

Table 18. Intention-to-treat analyses showing the influence of a 4-month interval aerobic training combined with strength exercise program compared to counselling on food intake and total Mediterranean Diet Score in climacteric women (n=150).

			Model I. Unadjusted				Model II. Adjusted for baseline values			
	Counselling Post-Pre (n=75)	**Exercise Post-Pre (n=75)**	**B**	**Confidence interval (95%) for B**	**β**	***P***	**B**	**Confidence interval (95%) for B**	**β**	***P***
Cereals, svgs/day	-0.117 (1.233)	-0.456 (0.936)	-0.339	(-0.694, 0.016)	-0.154	0.061	-0.148	(-0.417, 0.121)	-0.067	0.279
Non-refined cereals, svgs/day	0.095 (0.806)	-0.036 (0.704)	-0.131	(-0.376, 0.115)	-0.087	0.295	-0.104	(-0.308, 0.101)	-0.069	0.318
Potatoes, svgs/wk	-0.297 (1.431)	-0.380 (1.911)	-0.084	(-0.633, 0.466)	-0.025	0.764	0.173	(-0.160, 0.506)	0.051	0.307
Fruit, svgs/day	0.225 (1.003)	0.099 (1.336)	-0.126	(-0.511, 0.258)	-0.054	0.518	0.081	(-0.229, 0.391)	0.034	0.606
Vegetables, svgs/day	-0.354 (2.907	-0.463 (2.607)	-0.110	(-1.006, 0.787)	-0.020	0.809	0.038	(-0.524, 0.600)	0.134	0.893
Pulses, svgs/wk	0.049 (1.822)	0.077 (1.607)	0.028	(-0.530, 0.586)	0.008	0.921	-0.024	(-0.500, 0.452)	-0.007	0.920
Fish/shellfish, svgs/wk	-0.082 (3.326)	1.119 (2.610)	1.200	(0.231, 2.170)	0.198	0.016	1.110	(0.338, 1.883)	0.184	0.005
Red and cured meats, svgs/wk	-0.446 (4.682)	-0.594 (3.587)	-0.148	(-1.501, 1.205)	-0.018	0.829	0.154	(-0.837, 1.145)	0.019	0.759
Poultry, svgs/wk	0.307 (2.320)	0.376 (3.983)	0.070	(-0.993, 1.132)	0.011	0.897	0.304	(-0.562, 1.169)	0.047	0.489
Dairy products, svgs/day	-0.2583 (1.433)	-0.095 (0.946)	0.163	(-0.230, 0.557)	0.068	0.413	-0.026	(-0.340, 0.288)	-0.011	0.870
Whole-fat dairy products, svgs/day	-0.142 (0.851)	-0.107 (0.789)	0.035	(-0.232, 0.301)	0.021	0.796	0.020	(-0.173, 0.213)	0.012	0.837
Olive oil, svgs/day	-0.013 (1.232)	-0.244 (1.35)	-0.231	(-0.649, 0.188)	-0.090	0.278	-0.341	(-0.636, -0.046)	-0.132	0.024
Red wine, svgs/week	-0.278 (2.389)	-0.460 (3.426)	-0.182	(-1.144, 0.708)	-0.031	0.709	-0.133	(-0.665, 0.400)	-0.023	0.623
Beer, svgs/week	-0.539 (3.210)	0.923 (2.453)	1.462	(0.536, 2.389)	0.250	0.002	1.059	(0.369, 1.749)	0.181	0.003
Mediterranean Diet Score (0-55)	2.277 (4.616)	2.802 (4.618)	0.526	(-0.974, 2.027)	0.057	0.489	0.608	(-0.562, 1.779)	0.066	0.306

Mean results show the differences between post-pre intervention results for each variable with negative values as a reduction in the post evaluation compared to pre-evaluation; (standard deviation); β, standardized beta. Svgs, servings.

Table 19. Table 19. Per-protocol analyses showing the influence of a 4-month interval aerobic training combined with strength exercise program compared to counselling on food intake and total Mediterranean Diet Score in climacteric women (n=114) ¶.

			Model I. Unadjusted				Model II. Adjusted for baseline values			
	Counselling Post-Pre (n=55)	**Exercise Post-Pre (n=59)**	**B**	**Confidence interval (95%) for B**	**β**	***P***	**B**	**Confidence interval (95%) for B**	**β**	***P***
Cereals, svgs/day	-0,106 (1,254)	-0,412 (0,889)	-0.303	(-0.703, 0.097)	-0.142	0.136	-0.202	(-0.524, 0.120)	-0.094	0.217
Non-refined cereals, svgs/day	0,106 (0,851)	-0,084 (0,722)	-0.188	(-0.481, 0.105)	-0.120	0.206	-0136	(-0.397, 0.125)	-0.087	0.305
Potatoes, svgs/wk	-0.280 (1.429)	-0.345 (1.889)	0.012	(-0.628, 0.651)	0.003	0.972	0.155	('-0.252, 0.562)	0.046	0.453
Fruit, svgs/day	0,192 (0,941)	0,152 (0,917)	-0.014	(-0.366, 0.339)	-0.007	0.939	0.041	(-0.291, 0.374)	0.022	0.805
Vegetables, svgs/day	-0,456 (3,118)	-0,209 (2,200)	0.316	(-0.660, 1.292)	0.061	0.523	0.143	(-0.526, 0.813)	0.028	0.672
Pulses, svgs/wk	0,001 (2,006)	0,086 (1,666)	0.099	(-0.602, 0.801)	0.027	0.779	-0.027	(-0.650, 0.595)	-0.007	0.931
Fish/shellfish, svgs/wk	0,028 (2,934)	1,126 (2,490)	1.104	(0.113, 2.094)	0.206	0.029	1.047	(0.131, 1.963)	0.195	0.026
Red and cured meats, svgs/wk	0,0526 (4,143)	-0,477 (3,299)	-0.712	(-2.096, 0.672)	-0.097	0.310	-0.278	(-1.464, 0.907)	-0.038	0.643
Poultry, svgs/wk	0,398 (2,179)	0,378 (3,334)	-0.088	(-1.184, 1.008)	-0.015	0.874	0.088	(-0.943, 1.119)	0.015	0.866
Dairy products, svgs/day	-0,219 (1,502)	-0,067 (0,881)	0.194	(-0.249, 0.638)	0.083	0.387	0.044	(-0.345, 0.432)	0.019	0.824
Whole-fat dairy products, svgs/day	-0,141 (0,787)	-0,035 (0,500)	0.113	(-0.131, 0.358)	0.087	0.360	0.092	(-0.130, 0.315)	0.071	0.412
Olive oil, svgs/day	0.110 (1.197)	-0.180 (1.301)	-0.276	(-0.744, 0.192)	-0.111	0.244	-0.355	(-0.739, 0.029)	-0.143	0.070
Red wine, svgs/week	-0,559 (2,576)	-0,369 (3,255)	0.146	(-1.004, 1.297)	0.24	0.801	0.053	(-0.626, 0.733)	0.009	0.877
Beer, svgs/week	-0.324 (2.275)	0.921 (2.578)	1.039	(0.161, 1.917)	0.218	0.021	0.884	(0.118, 1.650)	0.186	0.024
Mediterranean Diet Score Total	2,208 (4,889)	2,657 (4,718)	0.470	(-1.328, 2.269)	0.049	0.605	0.574	(-0.925, 2.073)	0.060	0.450

Mean results show the differences between post-pre intervention results for each variable with negative values as a reduction in the post evaluation compared to pre-evaluation; (standard deviation); β, standardized beta; svgs, servings. ¶ Only women with available data and participants in the exercise group who attended ≥75% of the exercise sessions were included.

DISCUSSION

DISCUSSION

Summary of the main findings

Stage 1. Interval aerobic exercise combined with strength training on metabolically obese rats

The main findings of the present International Doctoral Thesis suggested that sedentary rats, regardless of their phenotype, presented worse body composition, glycaemic and lipid profile than the animals that performed the IASE protocol. Moreover, even with a genetically adverse metabolic profile, exercise clearly resulted on restoring insulin sensitivity to normal ranges. Furthermore, IASE protocol was associated with significant improvements in the cardiometabolic parameters analysed beyond CR, although best results were found when both interventions were combined. The IASE protocol decreased inflammation and promoted beneficial body composition (i.e. reduced fat mass and increased lean mass) and CR promoted better lipid profile. Moreover, even with a genetically adverse metabolic profile, IASE interacted for improving glycaemic profile to normal ranges, and in a higher degree than CR did. These findings suggest that IASE had greater overall cardiometabolic effectiveness than CR. Finally, despite CR promoted weight-loss in Zucker diabetic fatty rats, this effect was paralleled by a reduction in lean tissue.

Stage 2. Interval aerobic exercise combined with strength training on climacteric women

When transferring this animal study to a human model, the main findings suggested that a four-month IASE intervention decreased BMI and gynoid and android fat mass and increased BMC of the pelvis, compared to a counselling programme. Also, after the per-protocol analyses, a smaller reduction was shown in total BMC in the exercise compared to the counselling group, suggesting a potential clinical efficacy of the intervention to reduce the loss of total BMC. However, we observed no effects on other relevant markers of adiposity such as total fat mass, fat mass percentage, or visceral adipose tissue. The reductions found in gynoid and android fat mass were associated with lower

pharmacology expenditure. Moreover, results suggested that IASE could improve plasma glucose, lipid profile, CRP and systolic and diastolic blood pressure. However, lifestyle seminars (addressing the importance of a healthy diet and appropriate physical activity levels), might had also promoted improvements on these outcomes. Although greater improvements were observed after the exercise programme, few significant differences were found between groups, suggesting the important role of the diet during perimenopause. Indeed, when analysing dietary patterns, we found that women in the IASE group increased their beer consumption in one serving/week, which might be related to the social meetings between exercise mates after training.

Discussion of main findings with previous literature

Stage 1. Interval aerobic exercise combined with strength training on metabolically obese rats.

Study I. Effects of interval aerobic training combined with strength exercise on body composition, glycaemic and lipid profile and aerobic capacity of obese rats

To the best of our knowledge, the effects of IASE protocol had not been previously studied in obese animals. Several studies analysed the effects of exercise on body weight, fat mass, lipid profile or insulin sensitivity, however, most of them have used solely aerobic [13,14,36,37] or strength training[17,38,85,86]. Of the research focusing on aerobic training, the study by Haram et al.[13] 24 rats with a phenotype that closely resembles the metabolic syndrome were divided into three groups: continuous moderate exercise, interval aerobic training, or sedentary group. The authors observed an increase in high-density lipoprotein-cholesterol by the interval aerobic training whereas the other two groups remained unchanged. Contrary to our results, they found that both training protocols reduced triglycerides to an equal extent, whereas we did not observe decreases in triglycerides in our obese group. The absence of such differences could be explained by the severe hypertriglyceridemia that characterizes the obese Zucker rats[41,42].

Kim et al.[36] studied the effect of endurance training on glucose tolerance and body weight in Zucker rats. The animals ran in a treadmill during 60 min, 5 days/week. The obese training group decreased body weight and glucose tolerance. However, in contrast to our results, there was no body weight reduction in the lean Zucker rats, which could be due to the absence of strength training[17]. Cameron et al.[37] also investigated the effects of endurance exercise in a rat model of metabolic syndrome. According to our results, exercised rats showed decreased body weight and postprandial blood glucose. Exercise also improved plasma lipid profile, although contrary to our results Cameron et al.[37] failed to find effects on fasting glucose. Finally, Chang et al.[87] performed a moderate exercise protocol consisting in 60 min running at 20 m/min 7 days/week in 2 groups of obese and lean Zucker rats. Exercise reduced fasting glucose and insulin concentrations in the obese+exercise group, whereas in our study, fasting glucose was reduced in both lean and

obese exercise groups. The lower effects observed in glycaemic metabolism in the above-mentioned studies might be explained by the fact that the intensity of the running exercise performed was low (an adult rat can run until a velocity equal to ~60 m/min).

A different protocol was used by Teixeira de Lemos et al.[88] in which a group of lean and obese Zucker rats trained 3 days/week swimming for 60 min. A reduction of tumour necrosis factor alpha was observed in the obese+exercise rats. Similar results were obtained in our study but only in the lean+exercise group. Under our experimental design, interleukin-1 decreased 70% when the exercise was performed in the lean phenotype, although no reduction was observed in the obese phenotype. Finally, the protocol by Muhammad et al.[89] performed at a low intensity in rats did not improve metabolic syndrome markers.

Strength training may be a perfect complement in the clinical struggle against the metabolic syndrome[17,38] and some studies performed in rats were based uniquely on strength exercise[38,85,86]. Our group previously analysed the effects of hypertrophy strength training on body weight and plasma lipid profile[85]. In that study, the animals ran at 21 m/min, 5 days/week, with progressively increased weights in a bag tied with a cord to the tail. We found that body weight and plasma triglycerides were reduced and plasma high-density lipoprotein-cholesterol increased in the training group. That time we did not observe decreases in plasma total cholesterol like we did in the present study, which may be explained by the inclusion now of interval aerobic training. Donatto et al.[38] performed a training protocol where Wistar rats climbed a vertical ladder with weights secured to their tails. They found a reduction in glycaemia and low-density lipoprotein-cholesterol and an increase in high-density lipoprotein–cholesterol due to strength training as we but they did not find differences on triglycerides. Finally, they also demonstrated that strength training increased adiponectin levels and reduced interleukin-10 and TNF- α[38].

Other studies compared both types of training protocols in the same report (i.e. aerobic vs. strength exercise). Earnest et al.[24] examined the effects of aerobic, strength or aerobic+strength training for 9 months in patients with metabolic syndrome and type 2 diabetes. They observed a decrease in the metabolic syndrome prevalence after both training programs. They also found an association between these improvements and exercise efficiency, as measured by the maximal oxygen consumption. Under our experimental conditions, additional benefits were found on lipid and glycaemic profiles.

In addition to all the metabolic markers above mentioned, insulin resistance is thought to be essential in the development of the metabolic syndrome[90]. In this sense, Hall et al.[91] divided Type 1 diabetic rats into 5 groups; control, diabetic control, diabetic with strength training, and diabetic with high or low-intensity treadmill exercise. Strength trained rats climbed a ladder with incremental loads, while high or low-intensity trained rats ran on a treadmill at 27 or 15 m/min, respectively. They found that all exercise groups had lower glucose area under the curve than diabetic animals. Trained rats required lower insulin doses and a greatest reduction was evident in the high-intensity exercise group[91]. This supported the idea that high-intensity exercise programmes show greater improvements in insulin sensitivity than other types of exercise (i.e. moderate or light intensities). Indeed, we confirmed that the obese phenotype clearly presented insulin resistance (as indirectly measured through the oral glucose tolerance test and homeostatic model assessment-insulin resistance). Noteworthy, interval aerobic training combined with strength exercise interacted on reducing postprandial glucose 30, 60, 90 and 120 min after glucose intake.

Therefore, supported in our findings and the above-mentioned literature, we hypothesised that the use of a combined training protocol including aerobic and resistance training is more beneficial than other programmes that only focus in the development of a unique type of training.

The present data suggested that a combined training protocol including interval aerobic and strength exercise may be an effective therapy for obese individuals with metabolic syndrome, more especially if they have obtained no results on improving their glycaemic and lipid profiles with other treatments. We believed that the experimental conditions of our sedentary groups of either lean or obese rats could reproduce the amount of movement that is currently done by most of sedentary population and could constitute a reliable experimental model that can be directly extrapolated to the sedentary lifestyle of many people. Consequently, we suggested that interval aerobic training combined with strength exercise in the same work session might be a useful clinical tool in order to improve metabolic markers in obese individuals with metabolic syndrome.

Study II. Interval aerobic training combined with strength exercise improves metabolic markers beyond caloric restriction in Zucker rats

This study analysed the effects of CR and the most evidenced-based effective training protocol against obesity and the metabolic syndrome.

Several studies had analysed the effects of different exercise training protocols on body composition, lipid or glycaemic profile, although most of them used solely aerobic or strength exercise, as seen in the previous study. Furthermore, similar studies had compared the effects of CR and exercise on body composition and metabolic syndrome markers[10,11,26,33]. Larson-Meyer et al.[11] determined whether 25% CR or CR+exercise (12.5% CR+12.5% increase in strength exercise energy expenditure) lead to greater cardiometabolic benefits in overweight adults. Contrary to our results they found no differences between groups on body weight or fat loss (both groups reduced by ~10% body weight and by 25% fat mass). The lack of reductions on body weight in our IASE groups could be explained by the 8% increase in food intake in the exercise groups as well as by the strength-endurance component, with the consequent increases in lean mass[17] that we contrasted also through the higher quadriceps protein content. In agreement with our results both interventions improved HDL-C, though only the CR+exercise group showed significant reductions in LDL-C, total cholesterol and insulin sensitivity[11]. Importantly, our study included specific assessments of plasma phospholipids, which were significantly more reduced in the IASE compared to the CR groups (12% vs 7%). On a different note, we found no effect of IASE on plasma triglycerides which could be explained by the genetically characteristic extreme hypertriglyceridemia of the Zucker rat[42].

Morencos et al.[10] assessed a combination strength-aerobic exercise+CR in overweight adults. Such training promoted 10% weight-loss (22% under our experimental design). Fat percentage decreased by 12% (20% in our study), LDL-C was reduced by 11% (41% in our study), total cholesterol concentrations were 9% lower (22% in our study) and finally, they did not observe positive changes on plasma triglycerides or HDL-C whereas we have observed 38% lower triglycerides. Finally, Campbell et al.[34] compared the effects of CR plus interval exercise and CR plus continuous exercise on body composition and lipid profile in obese individuals. Both exercise groups resulted in improvements only

in very low LDL-C, without clear differences between the two exercise protocols. We hypothesize that the absence of differences between groups could be explained by the low sample size, or to the absence of strength training.

Besides the importance of the metabolic markers mentioned above, insulin resistance is thought to be critical to the development of metabolic syndrome[90]. In this sense, Coker et al.[33] examined the influence of CR vs exercise with and without weight-loss on insulin resistance in obese subjects. Exercise+weight-loss promoted the greatest improvement in glucose metabolism. Contrarily, we found that fasting glucose was 11% lower in the IASE groups, whereas CR did not play a significant role. Moreover, in our study the HOMA-IR index was doubly improved by the IASE than the CR (65 vs 31%, respectively). We speculated that the lower plasma adiponectin concentrations found in our CR groups could in part account for the lower glycaemic profile improvements observed[92,93].

Also in this line, Hall et al.[91] divided fifty rats into 5 groups; control, diabetic+sedentary, diabetic+strength, and diabetic high and low intensity treadmill exercise. All exercise groups resulted in lower intravenous glucose area under the curve than diabetic animals. Trained rats had the lowest insulin dose requirement, and the greatest reduction in insulin dosage was evident in high-intensity exercise. This supported the idea that high-intensity exercise interventions are required to obtain higher improvements on insulin sensitivity, and that while all exercise modalities can improve glucose tolerance, each mode leads to differential improvements on insulin requirements[91]. Therefore, our findings and previous literature suggested that the use of combined training protocols (i.e. interval aerobic training and strength exercise) could be more effective to improve glycaemic profile than other programs based on training of a single physical capacity (e.g. strength training). Moreover, our program was designed to train both capacities in the same 1h exercise session. Indeed, our group demonstrated in the previous study the effectiveness of this novel training protocol in obese versus lean animals for the first time. We found that IASE improved body composition, lipid and glycemic profiles, especially in obese rats. Future studies ought to determine whether this exercise intervention shows higher benefit in humans as compared with single-capacity training were warranted.

Remarkably, despite obesity is a major contributor of the leading causes of death in the world[28], a subgroup of obese people seem to be protected against obesity-related

metabolic complications. These individuals are described as metabolically healthy but obese[94]. Higher cardiorespiratory fitness should be considered a characteristic of the metabolically healthy but obese phenotype[95]. As expected, trained groups substantially improved cardiorespiratory capacity, which could be on the basis of the metabolic improvements observed. Of note, CR groups also showed an improvement in aerobic capacity, which may be associated with weight reductions promoted by such dietary intervention. Therefore, improvements in cardiorespiratory fitness might explain many of the benefits observed in both intervention groups, and the higher percentage of positive metabolic changes achieved by the exercise groups[96,97]. For instance, Earnest et al.[98] found a strong inverse relationship between maximal cardiorespiratory fitness and metabolic syndrome in almost forty thousand subjects. Adiposity reductions also mediate the observed positive metabolic changes[99]. Overall, our study in line with previous research, suggested that scientists, clinicians, and public health officials should focus on developing, testing, validating and, eventually, promoting the use of fitness-based interventions for obesity rather than focusing on weight-loss driven approaches to reduce cardiovascular diseases risk in obese individuals[96].

Stage 2. Interval aerobic exercise combined with strength training on climacteric women

Study III. Body composition changes in perimenopausal women following a concurrent exercise intervention: The FLAMENCO Project Randomised Controlled Trial

Similar to this study many others had analysed the changes in body composition after exercise in perimenopausal women and found comparable results. Even though body weight showed a meaningful reduction in the exercise group, we did not find significant differences between groups after the four-month intervention. Similarly, Maillard et al.[60] found no differences in body weight in neither high intensity nor moderate intensity four-month programmes, compared to baseline[60]. Contrary to these findings, the women in the study of Arsenault et al.[61] showed a significant reduction on body weight after an exercise programme, 3-4 times per week at a moderate intensity, during 6 months. Di Blasio et al.[62] also found weight differences between control and exercise groups after a 13-week low-intensity programme. These controversial results suggested the need of a large trial to understand the effects of different exercise intensities and types of training for weight loss in this population. Furthermore, dietary plus exercise interventions show greater positive effect on body composition than uniquely dietary or exercise-based programmes, especially regarding weight-loss and adiposity[100,101]. Notwithstanding, we aimed to isolate the exercise effect in order to explore the influence of this type of concurrent training on body composition in this specific population.

Despite the lack of clear significant differences in weight loss, we found a significant reduction in BMI in the exercise compared to the counselling group. These results were meaningful given that BMI is known to be a great tool to assess overweight and obesity[102], and menopause is associated with increased weight status and, consequently, higher risk of cardiovascular diseases[49].

Although we expected a clear lean mass increase in the exercise group, the results showed slightly greater increase in the counselling group. We hypothesized that the healthy lifestyle conferences given to the counselling group during the intervention period might had highly motivated the women to have a more active lifestyle. It is also worth noticing

that some data suggested that exercise programs resulted in a compensatory reduction in physical activity levels throughout the rest of the day[74,75]. This, together with the little differences found between groups of women meeting the minimum recommendations of physical activity, partially explained the weak improvements shown in body weight and fat mass in the exercise group. This hypothesis might, therefore, explained various of the null results observed in this study. Nevertheless, lean mass increased less than expected in the exercise group. As previous studies observed greater increases in lean mass following higher intensity[49] programmes, it was possible that the intensity or the length of the exercise programme in this study was insufficient to significantly increase lean mass.

Regarding fat mass, our moderate-vigorous programme resulted in a gynoid and android fat mass reduction in the exercise compared to the counselling group whereas no other fat measurements showed a significant reduction. Similarly to our results, Grossman et al.[63] did not find any reductions on fat mass measurements in either same length short-duration high intensity exercise or in their moderate exercise groups when comparing to baseline[63]. In the same line, Bouchonville et al.[103] did not find changes in visceral mass or overall body weight after one year of concurrent exercise. However, they did find differences, in line with what was previously stated[100,101], when exercise was combined with a dietary intervention[103], suggesting the high influence of diet on this kind of interventions. On the other hand, Mandrup et al.[49] found changes in all fat variables studied after a 3-month high-intensity training programme compared to baseline. Maillard et al.[60] found differences in total fat mass in both high-intensity and moderate-intensity programmes compared to baseline but only high-intensity interval training showed a reduction on abdominal and visceral fat. Therefore, higher intensity with interval training programmes should be considered for future projects, as well as the inclusion of dietary programmes for better results.

The exercise group showed no differences in total BMD, lumbar spine or pelvis BMD compared to the counselling group. Nevertheless, an increase in BMC of the pelvis was found in the exercise group. A healthier profile in total BMC in the exercise compared to the counselling group was also confirmed when conducting the per-protocol analyses. Despite the fact that this last result was not confirmed in the intention-to-treat analysis, it suggested a potential clinical efficacy of the exercise programme to reduce total body BMC loss. Other studies[66] had suggested that resistance training combined with high-

impact training improved BMD in the spine and femoral neck, while resistance training alone showed no differences[66]. Following this results, Heinonen et al.[67] did not find differences in BMD after resistance training. However, our concurrent exercise programme resulted in no changes on BMD of lumbar spine. Heinonen et al.[67] also found that an 18-month programme based on endurance training induced a significant trend indicating the maintenance of BMD in the distal radius and femoral neck. On the other hand, Wen et al.[68] did not find any differences between the exercise and counselling group in BMD after a 10-week programme based on step aerobic exercise. During perimenopausal years, bone loss is accelerated and even with exercise (especially regarding muscle strength) it may be difficult to load bones enough to prevent or retard bone loss during this critical transition period[65,104]. Therefore, we might state that, apparently, the length of the programme could be the reason of the little changes observed on bone in the present study, as changes in BMC and mostly BMD are long term.

To note is that we also observed that a reduction on gynoid and android fat mass, as seen after our exercise programme, impacted on pharmaceutical cost saving, and hence with health care costs. On the other hand, no association was seen between improved BMI nor BMC of pelvis with a reduced pharmaceutical expenditure. However, when analysing with per-protocol data, there was a between-group difference association with the overall pharmaceutical cost that showed a meaningful decrease in pharmaceutical expenditures on women that followed the training programme. We are aware that such a short period of time makes difficult to obtain strong evidence in pharmaceutical costs. However, we expected to observe some short-term influence of the exercise training programme on the consumption of anxiolytics, anti-inflammatories**,** analgesic, and relaxants. Therefore, this reduction in medication costs might probably be increased in the long term (more time for the physician to adjust pharmacology prescription after having more information regarding the evolution of the diseases plus more time to improve body composition through exercise). Future studies are warranted to confirm or contrast what type of exercise programmes might benefit perimenopausal women's body composition at the same time that could promote cost savings for the Health Systems. There are currently several studies analysing the association of different diseases such as cancer [105] or osteoporosis[106] with pharmaceutical costs, or analysing the costs of a specific drug on a similar populations to ours. However, as far as we know, the relationship between changes in body composition and pharmaceutical costs had not been addressed. The

present study showed novel and useful data and its findings should increase interest in finding exercise programmes that can promote improvements on body composition with parallel reductions on pharmaceutical costs. This should also lead to future studies with a bigger sample size to verify the present results.

Study IV. Effects of concurrent exercise on cardiometabolic status during perimenopause. The FLAMENCO Project

The role of exercise on cardiometabolic markers has shown controversial results, probably due to the heterogeneity of the studies. Although the present study strictly followed the Consort guidelines for Randomised Controlled Trials during the entire intervention, the inclusion of a counselling group instead of a control group for ethical reasons may be the main reason for the weak results found in this regard.

Other studies had seen similar improvements in blood glucose after Nordic Walking combined with a 1,500 kcal diet, showing better results than with diet alone[107] in overweight and obese postmenopausal women. The same trend was observed after a 6-week resistance training in postmenopausal women[108]. Furthermore, the LDL-C reduction observed in our exercise group matches a review of previous studies[109], as diet alone was not as efficient in the reduction of LDL-C. Similarly, the borderline significance observed in HDL-C, increasing in the exercise and decreasing in the counselling group, also concured with previous findings[109,110]. This highlights the importance of an exercise-based plan, as the diet counselling alone could not attenuate the usual reduction found in HDL-C during menopausal transition[52]. Therefore, future studies analysing the benefits of a combined counselling plus exercise intervention during perimenopause may lead to interesting and complementary findings.

Furthermore, we speculated that the significantly greater improvements seen in plasma glucose and CRP in the counselling group compared to the exercise group could be related to the diet and active lifestyle conferences, which we believed were very influential. Controversial results showed improvements on CRP when combining aerobic exercise with a weight loss diet in overweight/obese and aerobic exercise alone in postmenopausal women[59,111]. On the other hand, no variations had been observed on CRP levels neither in postmenopausal women after resistance training[58] nor in healthy men after resistance training, aerobic training, or concurrent training[112]. It should be taken into account that improvements seen after an exercise intervention were usually more evident in overweight, dyslipidaemia or hypertensive individuals, among other complications[57], while our sample of participants was overall healthy.

The increase on triglyceride levels was the only value in this study that worsened in both counselling and exercise groups. These findings were not consistent with most studies,

which show decreases[69,113] or no effects[53] on triglycerides after an exercise programme. Hence, we speculated that this divergence could be due to a reduction of the fasting hours during the summer season when the post evaluation was conducted.

The lack of differences found between both groups could have various interpretations. First, it is important to consider the big loss of blood samples that this study experienced. Despite the fact that per-protocol analyses were conducted, the small final number of plasma samples could be one of the main reasons for the lack of significance in some outcomes. For this reason, supplementary analyses showed intention-to-treat analyses, where similar tendencies for all variables were found. As previously stated, the improvements on plasma glucose and CRP may had been greater if the participants had been metabolically unhealthy. Moreover, we believed that most of the changes observed in the counselling group were due to a high and unexpected acceptance and follow-up of the lifestyle conferences. The lecturers provided participants with professional advice on how to improve their diet according to the Mediterranean Dietary pattern, as well as insisting on the importance of having an active behaviour. We were aware that women in the exercise group started meeting after their training sessions with their new colleagues of the research study. We believe this social behaviour implies changes in the dietary habits that might have attenuated the potential improvements expected on plasma glucose and lipid profile with the exercise training programme.

With regard to this hypothesis, a previous meta analytic work already stated that dietary interventions have a greater influence on improving lipid levels than exercise, although a combination of both would be the most influential[113]. In line with these explanations, a review of randomised controlled trials concluded that exercise plus diet seemed to have an additive effect on improving lipid profile and hypertension rather than exercise alone[57]. Therefore, future studies should combine this concurrent exercise protocol with dietary interventions in search of better results.

Study V. Influence of participating in a group exercise program on dietary behavior. The after-training beer phenomena. Findings from the FLAMENCO Project

Although several studies had compared the effects of exercise, diet, and the combination of both interventions in various physiological variables in populations of older adults and postmenopausal women[101,103,109,111,113], the importance of analysing possible non-controlled dietary pattern changes in response to exercise was key when studying those variables. To the best of our knowledge, no previous study had checked the effects of following an exercise program on the dietary habits of perimenopausal women. Only few studies had approached this issue in other populations despite of its relevance when it comes to interpreting the exercise intervention results. Donnelly et al.[114], studied the self-selected energy intake in several occasions while exercising, finding no changes after 16 months of a moderate exercise protocol (5 days/week) in young adults, nor after 8 months of brisk walking (5 days/week) in overweight women[115]. In a more recent study, Rocha et al[116], also found no changes in energy intake after 12 weeks of moderate intensity training in men. Furthermore, a study analysing 20 postmenopausal women undergoing 8 weeks of moderate intensity training, found no differences in energy intake between exercise and control groups[117]. However, the studies performed by Donnelly et al.[118] and Wood et al.[119] observed a reduction of fat intake after 18 months and 1 year of moderate training in moderately obese women and overweight men respectively[118,119], and another study from Wood et al.[120] also found a reduction of fat intake with an increase in carbohydrate intake after 2 year moderate intensity exercise in middle-aged men[120].

All these previous studies confirmed our results, however, the significant increase we found in beer intake had no precedents. Although it is known that exercise can occasionally increase uncontrolled eating and food cravings[116], we believe the origin of this change in this specific study has another explanation. Also, women going through menopause period tend to increase their alcohol consumption and it is linked to stress and depression related to menopause[121]. However, the characteristics of these specific change along the intervention led us to hypothesize that these new patterns were related to the social meetings that occurred among the exercise group, that our research team could corroborate, but not control. Concerning the increase on fish intake we did not find studies supporting this change, however, we also related it to the consumption of beer and the meetings, as it is often seen in the South of Spain to be given free food with the beer, and it is commonly fish. Although that seemed to be an improving dietary pattern, further

information about how the fish was cooked would have been appreciated as restaurants often serve it fried when accompanying the beer. For this reason, future studies should use a 24h recall to have a more detailed and precise record of the dietary patterns.

Regarding to mental health, menopause women are more likely to suffer from loneliness, related to low psychological scores and partner stability and relation[122]. Furthermore, 50% of the exercise group were housewife at the time of the study intervention, which may occasionally give women less possibilities to connect with people other than their close relatives, unless they are involved in recreational activities.

Furthermore, in agreement with previously studies on exercise and its physiological response on mental health[123–125], the results from our project suggested improvements on quality of life, anxiety and mental health, that will be further explored in future studies. For all these, we believed the exercise program contributed in the improvements of women's life, not only as a physical health intervention as we were firstly looking for, but also as a mental therapy against loneliness and probably promoting other mental health benefits.

This would give a notable importance of exercise (as practiced in groups of the same age range) on mental health, and we encourage future studies to work in this promising hypothesis.

We are confident that knowing whether perimenopausal women following a training program tend to change into a healthier dietary habit or on the other hand, are more permissive with their meals after burning calories with exercise. Therefore, future exercise studies should consider tracking their participants diet, or even follow a dietary intervention. In this manner, less unexpected changes on cardiometabolic outcomes may be found when analysing exercise groups.

LIMITATIONS AND STRENGTHS

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Limitations

Study I and II. Some limitations of the present study need to be mentioned: First, the current physiological results obtained in rodents must be confirmed in humans and cannot be directly extrapolated. Second, it is relevant to consider that the trainability of both phenotypes is not the same. Obese phenotype rats have greater difficulties for training and the velocity and total work volume were smaller, which may affect the potential improvements. Finally, we have not compared our training protocol with other exercise protocols (e.g. compared with only resistance training).

Study III, IV and V. For ethical reasons, the control group received the previously stated conferences. Consequently, we lacked a control group with no stimulus at all with which we could compare the real efficacy of our intervention. Hence, results should be interpreted with caution. Although the counselling women did not participate in the exercise intervention, the conference about increasing physical activity to improve their health, life expectancy, prevent pathologies, and the dietary recommendations base on the Mediterranean Diet may have been more influential than initially expected.

Women included in the studies were not particularly affected by menopause (mild severity) and showed moderate-high physical activity levels, which hinder the extrapolation to women severely affected by menopause, or with poor physical activity levels. Secondly, this study lacks plasma hormonal analyses to objectively assess menopause status (e.g. oestrogens, follicle stimulating hormone, and luteinizing hormone).

In *study III* more specific bone analyses would have added relevant information to the current study (i.e. vertebral and femoral neck specific scanners).

In *study IV,* the big loss of blood data made an important reduction of the sample for the statistical analysis. Finally, we could not control the meetings after the training sessions of the women in the exercise group with their new colleagues of the research study.

These studies have evaluated many outcomes, and some of the associations may be due to chance. Thus, caution is advised in the interpretation of the results and replication in

other studies.

In *study V* the measurement of dietary patterns as done with the food frequency questionnaire is less precise than de 24-hour dietary recall, that would be better for future studies focusing on this topic.

Strengths

Study I and II. The genetically obese Zucker rat is a model of metabolic syndrome that shares many similarities with humans with this condition, and this experimental model also allowed control of food intake restriction in an objective, quantifiable way, which is difficult in humans. Moreover, these studies assessed the common and independent influence of IASE and CR on metabolic markers in rats with or without an adverse phenotype, which allowed us to compare the effectiveness such interventions, and analyse their potential interactions.

Study III, IV and V. The measurement tool employed to assess body composition (i.e. DXA) is widely valid and reliable, thus the accuracy of the results is warranted. Also, the studies show intention-to-treat analyses, those being potentially important as they replicate how this kind of programmes would work in real life. Per-protocol analyses were also included, being able to isolate and evaluate the clinical efficacy of the present concurrent exercise intervention.

It is also important to highlight that the design of this study as a randomised controlled trial is considered the gold standard for evaluating efficacy in clinical research. Furthermore, this randomised controlled trial was strictly supervised during all stages of the study, and the intensities of the training sessions were monitored periodically.

CONCLUSIONS/CONCLUSIONES

CONCLUSIONS

General conclusion

The findings of the present International Doctoral Thesis suggest that an interval aerobic training combined with strength exercise intervention carried out in obese rats with the metabolic syndrome had a relevant influence on the cardiometabolic profile of the animals towards a healthier status. The effects of the same protocol in human participants with a predisposition to cardiometabolic abnormalities (i.e. climacteric women) also showed a tendency to a healthier cardiometabolic status but with weaker results, especially regarding body composition, lipid profile and glucose. These attenuated results might be explained by changes in the dietary social patterns linked with the exercise program. This confirms the idea of the strong need of verifying results gotten with animal models and transferring the studies to human contexts. Future studies should combine this concurrent exercise protocol with dietary interventions based on the Mediterranean Diet in search of better results.

Specific conclusions

Stage 1. Interval aerobic exercise combined with strength training on metabolically obese rats.

1. Interval aerobic training combined with strength exercise reduced body weight, fat mass, fasting and postprandial glucose, insulin and homeostatic model assessment-insulin resistance and improved lipid profile.

2. Even in an obese phenotype, the practice of this type of concurrent exercise may enhance body composition and lipid profile and restore glucose control to normal ranges.

3. Interval aerobic training combined with strength exercise improved inflammation and glycaemic profile and body composition beyond caloric restriction.

4. Applying a combined exercise-diet intervention may be effective to enhance metabolic status without leading to weight regains after dietary interventions based uniquely on caloric restriction.

Stage 2. Interval aerobic exercise combined with strength training on climacteric women.

5. Interval aerobic training combined with strength exercise reduced body mass index and gynoid and android fat mass, and improved bone mineral content of the pelvis, but had no effects on other relevant markers such as body weight or lean mass.

6. A reduction in gynoid and android fat mass, as seen after our training programme, was associated with lower pharmaceutical expenditure.

7. Interval aerobic exercise combined with strength exercise programme promoted a healthier cardiometabolic profile.

8. A healthier diet was simply implemented through four conferences in the counselling group. Women in the exercise group increased their beer consumption, which might have been due to the social meetings after the exercise trainings.

9. While working with an animal model completely allowed the researcher to control many potential confounders, human participants changed some of their dietary patterns that might have influenced results.

CONCLUSIONES

Conclusiones generales

Los resultados de la presente Tesis Doctoral Internacional sugieren que el protocolo de entrenamiento aeróbico interválico combinado con fuerza llevado a cabo en ratas Zucker genéticamente obesas tiene efectos beneficiosos para su salud a nivel cardiometabólico. Los efectos del mismo protocolo de entrenamiento sobre los participantes humanos con predisposición a la obesidad (mujeres en etapa climatérica), también resaltaron una tendencia hacia un perfil cardiometabólico más saludable, aunque los resultados obtenidos fueron menos consistentes especialmente en lo que referido al peso y a la masa magra, el perfil lipídico y la glucosa en plasma. La debilidad de estos resultados parece estar relacionada con un cambio en los patrones de vida de las mujeres, tanto en la dieta como en la actividad física, variables que no se pudieron controlar en el estudio. Esto confirma la idea sobre la gran necesidad de contrastar los resultados obtenidos en estudios con animales con estudios llevados a cabo en situaciones reales en humanos. Futuros estudios deberían combinar el ejercicio aeróbico con intervenciones dietéticas basadas en la dieta mediterránea, en busca de una mejora de los resultados.

Conclusiones específicas

Etapa 1. Entrenamiento aeróbico interválico combinado con fuerza en ratas metabólicamente obesas.

1. El entrenamiento aeróbico interválico combinado con fuerza redujo el peso corporal, la masa grasa, glucosa en ayunas y postprandial, la insulina y el índice HOMA de los animales y mejoró el perfil lipídico.

2. Incluso en un perfil de fenotipo obeso, la práctica de ejercicio mejoró la composición corporal, el perfil lipídico, pudiendo restablecer incluso los niveles de glucosa.

3. El entrenamiento aeróbico interválico combinado con fuerza mejoró el perfil

inflamatorio y glucémico y la composición corporal por encima de la restricción calórica.

4. Aplicar una intervención de ejercicio y dieta combinados puede mejorar el estado metabólico sin llevar a ganancias de peso después de la intervención, como puede pasar después de las intervenciones basadas solamente en la restricción calórica**.**

Etapa 2. Entrenamiento aeróbico interválico combinado con fuerza en mujeres en etapa climatérica.

5. El entrenamiento aeróbico interválico combinado con fuerza redujo el índice de masa corporal, la masa grasa ginoide y androide y mejoró el contenido mineral óseo de la pelvis, pero no tuvo efectos sobre otras variables de la composición corporal como el peso corporal o la masa magra.

6. Una reducción en la masa grasa ginoide o androide (como la presente después de aplicar el programa de entrenamiento concurrente), se asoció con una reducción en los gastos farmacológicos de las mujeres.

7. El entrenamiento aeróbico interválico combinado con fuerza promovió un perfil cardiometabólico más saludable.

8. Las cuatro conferencias dadas al grupo consejos tuvieron un impacto en forma de dieta más saludable. Las mujeres del grupo de ejercicio incrementaron su consumo de cerveza en, aproximadamente, una ración por semana, lo cual se ha relacionado con los encuentros post-ejercicio que empezaron a suceder entre las mujeres del grupo.

9. Mientras en el estudio llevado a cabo en un modelo experimental animal se pudo tener un control total sobre muchas potenciales variables contaminantes, el programa de ejercicio llevado a cabo con mujeres en etapa climatérica indujo cambios en los patrones alimentarios de éstas, lo que muy probablemente tuvo un papel relevante sobre las variables analizadas.

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SHORT CURRICULUM VITAE

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