Natriuretic peptides as a possible mediator of positive effects of exercise training in patients with the metabolic syndrome:

a randomized controlled clinical trial.

FINAL DEGREE PROJECT

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1. ABSTRACT.

**Background:** Natriuretic Peptides are a group of peptide-hormones that are well known for their role in cardiovascular homeostasis. During the last decade, a wide range of novel findings about natriuretic peptides proves that NPs have also an active role in the defense against metabolic disease. NPs have been found to prevent the accumulation of fatty acids, to favorably affect adipose tissue distribution and to increase the basal metabolism trough many mechanisms. On the other hand, evidence has shown obesity and insulin resistance to be associated with lower levels of circulating natriuretic peptides. Thus, people with this metabolic profile, as patients with metabolic syndrome, could be stuck in a vicious cycle in which a suppressed natriuretic peptide system leads to the impairment in the ability to resist fat accumulation and insulin resistance. Therefore, any measure that could help to increase circulation and action of NPs could help to break this cycle, improving the metabolic profile. Fortunately, a number of novel stimuli have been described like weight loss or exercise, suggesting the possibility that the natriuretic peptide system could contribute to the favorable metabolic effects of exercise.

**Objective:** the main objective of this study is to evaluate if a 2-month moderate-intensity exercise training intervention leads to an increase in NT-ProBNP circulating levels in patients with metabolic syndrome and a sedentary lifestyle in order to study if positive effects of exercise training in metabolic syndrome may partially due to Natriuretic Peptides actions.

**Methods:** This study will be a controlled, randomized and single-center clinical trial. It will be an open study, but observer blind will be done. This study will be set in Hospital Santa Caterina, in the Sports Medicine Department and patient sampling will take place in the CAP of the 4 ABS of the city of Girona. The recruitment will be based on a consecutive non-probabilistic sampling method. The participants will be 66 patients (from 18 to 65 years old) with metabolic syndrome, defined as the current criteria of the NCEP ATP III, and a sedentary lifestyle at baseline. The patients will be randomly divided in two groups of 33 patients (1:1). The first group will go under a 2-month moderate-intensity exercise training program and the second group will be the control group that will maintain their baseline sedentary lifestyle. The main dependent variable will be the increase in NT-proBNP blood circulating levels in the exercise group, comparing to the control group, and it will be measured at baseline and at the end of the intervention by electrochemiluminescence immunoassay. Secondary dependent variables will be the metabolic syndrome components, the insulin resistance condition and the subjects cardiovascular fitness. The results will be expressed as percentages for categorical variables and as the mean±standard deviation for continuous variables. A bivariate analysis will be also performed using a Student test to compare continuous variables and a Chi² test for categorical variables.

**Key words:** Natriuretic peptides, metabolic syndrome, exercise training, insulin resistance, obesity.
2. ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
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<tbody>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>DM2</td>
<td>Diabetes mellitus type 2</td>
</tr>
<tr>
<td>MS</td>
<td>Metabolic Syndrome</td>
</tr>
<tr>
<td>CPR</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>PAI-1</td>
<td>Plasminogen activator inhibitor-1</td>
</tr>
<tr>
<td>IR</td>
<td>Insulin resistance</td>
</tr>
<tr>
<td>HOMA</td>
<td>Homeostasis model assessment</td>
</tr>
<tr>
<td>NPs</td>
<td>Natriuretic Peptides</td>
</tr>
<tr>
<td>ANP</td>
<td>Atrial Natriuretic peptide</td>
</tr>
<tr>
<td>BNP</td>
<td>Brain natriuretic peptide</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>N-terminal pro-B-type natriuretic peptide</td>
</tr>
<tr>
<td>NPRA</td>
<td>Natriuretic peptide receptor A</td>
</tr>
<tr>
<td>NPRC</td>
<td>Natriuretic peptide receptor C</td>
</tr>
<tr>
<td>HDL</td>
<td>High-density lipoproteins</td>
</tr>
<tr>
<td>CAP</td>
<td>Centro de Atención Primaria</td>
</tr>
<tr>
<td>ABS</td>
<td>Área básica de Salud</td>
</tr>
<tr>
<td>NCEP</td>
<td>National Cholesterol Education Program</td>
</tr>
<tr>
<td>IDF</td>
<td>International Diabetes Federation</td>
</tr>
<tr>
<td>SEEN</td>
<td>Sociedad Española de Endocrinología y Nutrición</td>
</tr>
<tr>
<td>RPE</td>
<td>Relative Perceived Exertion</td>
</tr>
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</table>
3. INTRODUCTION

3.1. Metabolic syndrome.

3.1.1 Definition. (2–4)
The metabolic syndrome consist on the co-occurrence of many risk factors for type 2 diabetes mellitus and cardiovascular disease. These factors include dyslipidemia (raised triglycerides and lowered high-density lipoprotein cholesterol), raised blood pressure, raised fasting glucose, and central obesity. In addition, persons with these characteristics commonly manifest a prothrombotic state and a proinflammatory state.

The metabolic syndrome is not an absolute risk indicator, because it does not contain many of the factors that determine absolute risk, for example, age, sex, cigarette smoking, and low-density lipoprotein cholesterol levels. Nonetheless, patients with the metabolic syndrome are at twice the risk of developing CVD over the next 5 to 10 years as individuals without the syndrome(2). At a clinical level, individual patients with the metabolic syndrome need to be identified so that their multiple risk factors, including lifestyle risk factors, can be reduced.

There are several definitions for the metabolic syndrome, leading to some difficulty in comparisons from studies using different criteria (see annex 1). This has also led to some confusion on the part of clinicians regarding how to identify patients with the syndrome. The National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) criteria is the most widely used (5). Another point that has been criticized is the inclusion of patients with clinical cardiovascular disease (CVD) or diabetes as part of the syndrome that is intended to define risk for these illnesses. (4)

In the NCEP ATP III classification, the evidence of insulin or glucose abnormalities is not required, although abnormal glycemia is one of the criteria. In contrast, in the WHO classification and the EGIR criteria for the syndrome, glucose disorders are the main requirement. On the other side, the IDF criteria consider the central obesity, measured by the waist circumference, as an essential component in the metabolic syndrome definition (2). ATP III metabolic syndrome criteria were updated in 2005 in a statement from the American Heart Association (AHA)/National Heart, Lung, and Blood Institute (NHLBI) (3). Current ATP III criteria defined the metabolic syndrome as the presence of any three of the following five traits:

- Abdominal obesity defined by waist circumference: ≥102 cm in men and ≥88 cm in women, except for patients from South Asia, Japan and China which appropriate threshold is ≥90 cm in men and ≥80 cm in women.
- Altered fasting plasma glucose: ≥100mg/dL or drug treatment for elevated blood glucose.
- Blood pressure ≥130/85 or drug treatment for elevated blood pressure.
- Low serum high-density lipoproteins (HDL) cholesterol: <40mg/dL in men and <50mg/dL in women or drug treatment for low HDL cholesterol.
- High serum triglycerides: ≥150mg/dL or drug treatment.
3.1.2. Prevalence.

The prevalence of the metabolic syndrome, as defined by the 2001 Adult Treatment Panel III (ATP III) criteria, was evaluated in 8814 adults in the United States (men and women aged 20 years or older) participating in the third National Health and Nutrition Examination Survey (NHANES III, 1988 to 1994), a cross-sectional health survey of a nationally representative sample of civilian US population. (6)

The overall prevalence was 22 percent, with an age-dependent increase (6.7, 43.5, and 42.0 percent for ages 20 to 29, 60 to 69, and >70 years, respectively). Mexican Americans had the highest age-adjusted prevalence (31.9 percent). Among African Americans and Mexican Americans, the prevalence was higher in women than in men (57 and 26 percent higher, respectively).

Figure 1: Age-Specific Prevalence of the Metabolic Syndrome Among 8814 US Adults Aged at Least 20 Years, by Sex, National Health and Nutrition Examination Survey III, 1988-1994. Extracted from (6).

The metabolic syndrome prevalence has changed since this study was published and it is becoming enormously common. The Data from NHANES 1999 to 2000 demonstrate that the prevalence has continued to increase, particularly in women. The age-adjusted prevalence was 27.0% and the age-adjusted prevalence increased by 23.5% among women and just 2.2 % among men. (7)

What is more, using data from the NHANES 1999 to 2002 database, 34.5 percent of participants met ATP III criteria for the metabolic syndrome compared with 22 percent in NHANES III (1988 to 1994) (8)

However, the prevalence of the syndrome depends on the classification used. Many studies have compared differences between many definitions using the same database. Using data from the National Health and Nutrition Examination Survey (NHANES) 1999 to 2002 database (a total of 3,601 men and women aged ≥20 years), 39 percent (39.9 among men and 38.1 among women) of United States adult participants met IDF criteria for the metabolic syndrome, compared to 34.5 percent (33.7 among men and 35.4 among women) using the ATP III criteria. The two definitions overlapped for 93 percent of subjects in determining presence or absence of the metabolic syndrome. (8)

To sum up, in the U.S, using the IDF definition of the metabolic syndrome leads to a higher prevalence of the metabolic syndrome than the estimated based on the NCEP definition, in all of the demographic groups, especially among Mexican-American men.
Comparing to US prevalence of metabolic syndrome, in Spain prevalence of this syndrome seems to be lower. According to the MESYAS Registry, that collected data from 7256 individuals actively employed, the prevalence was 10.2%, according to modified ATP-III criteria. It was significantly higher in men than in women (8.7% and 3% respectively). All the components were more common in men than in women, except HDL-cholesterol level. Prevalence increased with age, male gender, hypertension, diabetes and obesity. (9)

3.1.3. Etiopathogeny and underlying risk factors.

The main underlying risk factors for the syndrome appear to be:

- **Weight.** Increased body weight is a major risk factor for the metabolic syndrome. In NHANES III, the metabolic syndrome was present in 5 percent of those at normal weight, 22 percent of those who were overweight, and 60 percent of those who were obese.(10)

However, independently of the weight, **abdominal obesity**, normally measured by waist circumference, is a strong predictor of the metabolic syndrome. Moreover, abdominal or upper-body obesity (intraperitoneal or visceral fat and subcutaneous fat) correlates strongly with **insulin resistance**, specially visceral type. (11) An increase in the visceral abdominal fat leads to a Chronic proinflammatory state in which proinflammatory adipokines, that induce insulin resistance, are continuously released (IL-1,6,18, resistin, TNF-α, CPR). What is more, hormones that increase insulin sensitivity, as the adiponectin, decrease.

As a result, there is an increase in the fatty acid release from the adipocyte to the blood. This fact leads to the formation of liver, heart, muscle and β cell pancreas depots having a lipotoxic effect in these organs, which enhance the metabolic problems. While pancreas is functional, the insulin resistance is compensated with hyperinsulinemia until pancreas fail and prediabetes or DM2 appear.

On the other hand, obesity also leads to the continuous release of prothrombotic factors as the PAI-1.(3,12)

- **Insulin resistance.** IR is characterized by a diminished response to the biological effects of insulin in the different target tissues. As a compensatory mechanism, more insulin is produced (hyperinsulinism), and blood glucose levels can remain in a normal threshold. When the pancreas is not able to compensate the insulin resistance, glucose abnormalities appear leading to prediabetes or DM2. As it was explained before, IR is associated with obesity, predominantly abdominal distribution of fat, elevated triglyceride levels, low HDL cholesterol, small LDL particle size and elevations in inflammatory cytokines, being a basic component of the syndrome. Several ways of measuring IR has been proposed. The HOMA (homoeostasis model assessment) index is one of the most used methods (13) and it quantifies insulin resistance and beta-cell function. The steady-state basal plasma glucose and insulin concentrations are determined by their interaction in a feed-back loop.

The HOMA authors used data from physiological studies to develop mathematical equations describing glucose regulation as this feedback loop. They published computer software that solves the equations, in order to estimate insulin resistance and β-cell function, from fasting glucose and insulin levels.
They also published an equation in order to make this estimation available in the clinical practice. This equation has several modalities. In our study we will use:

Fasting glucose (mg/dL) X fasting insulin (U/mL)/405.

However, the estimation with the computer software is more accurate.

- **Other factors:** older age, race (highest in Mexican American and lowest in Afroamerican), postmenopausal status, smoking, low household income, high carbohydrate diet, alcohol consumption, and physical inactivity, soft drink and sugar-sweetened beverage consumption (10) and a parental history of metabolic syndrome. (14)

### 3.1.4. Therapy.

The metabolic syndrome is an important risk factor for the development of type 2 diabetes, cardiovascular disease (CVD) and other associated disorders. Thereby, If we identify those patients with metabolic syndrome, who needs aggressive lifestyle modification focused on weight reduction and increased physical activity, the risk of developing the related disorders would be reduced(3). In fact, this syndrome may be a reversible condition if addressed early on.

The two main goals of metabolic syndrome are: (3,5)
- To treat underlying causes (as overweight/obesity and physical inactivity) by intensifying weight management, controlling diet and increasing physical activity.
- To treat cardiovascular risk factors if they persist despite lifestyle modification.

Aggressive lifestyle modification focused on weight reduction, diet and increased physical activity is the essential therapy for the management of metabolic syndrome.

Long-term adherence to these changes is essential for the successful management of this chronic condition. What is more, long-term engagement in lifestyle changes may result in its resolution. (15)

**1. Weight reduction** is optimally achieved with a multimodality approach including diet, exercise, and possible pharmacologic therapy, as with orlistat (16).

Weight reduction has been the main goal of most intervention studies on patients with the metabolic syndrome because it likely activates one or more underlying mechanisms that lead to improvements in the metabolic syndrome profile (15). Weight loss is associated with significant improvements in blood glucose control and lipid and non-lipid abnormalities, as well as with metabolic syndrome resolution. Even a moderate weight loss (around 7%) has resulted in substantial reductions in blood pressure, glucose, triglyceride, and total cholesterol concentrations within a four-week period, despite the potential persistence of an elevated body mass index (BMI) (even >38 kg/m2). Usually, but not always, weight reduction is also associated with improvements in inflammation related markers as adiponectin and tumor necrosis factor-alpha concentrations.

Obviously, as greater the reduction in BMI is, more improvements are likely to be observed.
2. Diet: Several dietary approaches have been recommended:

**- The Mediterranean diet:** (17–19). The Mediterranean diet (MD) is a dietary pattern with cardio protective effects. It is composed by high consumption of monounsaturated fatty acids (olives and olive oil) and daily consumption of fruits, vegetables, whole grain cereals, low-fat dairy products and moderate consumption of wine, normally with meals. It also includes weekly consumption of fish, poultry, tree nuts, and legumes. The red meat consumption is low, like twice/month. Compared with a low-fat, prudent diet, this diet has shown to get greater improvement on metabolic syndrome parameters, even after controlling for weight loss.

**- The Dietary Approaches to Stop Hypertension (DASH) diet** (daily sodium intake limited to 2400mg, and higher in dairy intake than the Mediterranean diet), compared to a weight reducing diet emphasizing healthy food choices, resulted in greater improvements in triglycerides, diastolic blood pressure, and fasting glucose, even after controlling for weight loss. (20)

**- A diet that is low in glycemic index/glycemic load,** replacing refined grains with whole grains, fruits and vegetables, and eliminating high-glycemic beverages, may be particularly beneficial for patients with the metabolic syndrome. (21)

3.2 Exercise and fitness.

A large body of observational data shows an association between higher levels of physical activity and lower rates of many chronic diseases. In contrast, physical inactivity is a component of reduced life expectancy. (22)

3.2.1 Definition and terms: (23)

- **Physical activity:** is any body movement produced by skeletal muscles that results in energy expenditure beyond resting expenditure.

- **Exercise:** is the part of physical activity that is planned, structured and repetitive. It has de purpose of improvement or maintenance of physical fitness. Physical fitness is a set of attributes related to the ability to perform physical activity. This attributes consist of cardiorespiratory fitness, muscle strength, body composition, and flexibility.

When defining the amount of physical activity or exercise, we can measure the dose and the intensity of activity (See annex 2).

- **Dose** refers to the total amount of energy expended in physical activities that require repetitive muscular movement (usually expressed in kilojoules or kilocalories).

- **Intensity** reflects the rate of energy expenditure during such activity. It can be defined in absolute or relative terms:

  - **Absolute intensity:** reflects the rate of energy expenditure during exercise and is usually expressed in metabolic equivalents or METs, where 1 MET equals the resting metabolic rate of 3.5 mL O2/kg of corporal weight/min.
Relative intensity refers to the relative percentage of maximal aerobic power that is maintained during exercise and is expressed as percentage of maximal heart rate or percent of VO2max.

Maximal oxygen consumption (VO2 max) is the greatest amount of oxygen a person can take in from inspired air while performing dynamic exercise involving a large part of total muscle mass. It is considered the best measure of cardiovascular fitness and exercise capacity. VO2 max represents the amount of oxygen transported and used in intracellular metabolism. It is the product of cardiac output and systemic arterio-venous oxygen difference. VO2 max is influenced by age, sex, exercise habits, heredity, and cardiovascular clinical status.

- **Moderate-intensity activities**: are those performed at a relative intensity of 40% to 60% of VO2max (or absolute intensity of 4 to 6 METs).
- **Vigorous-intensity activities**: are those performed at a relative intensity of >60% of VO2max (or absolute intensity of >6 METs). For example, brisk walking at 4.8 km/h (>4METs) is considered, in relative terms, light for a 20-year-old healthy person but represents a vigorous intensity for an 80-year-old person.

What is more, to measure the relative intensity of exercise, apart from objective measures as the heart rate or the VO2 max percentage, some subjective scales can complement information. The Borg scale of perceived exertion measures the subjective rating of the intensity of exertion perceived by the person exercising and it is a sound indicator of relative fatigue. It is a 6 to 20 grade scale where >18 indicates the patient has performed maximal exercise and >15-16 suggest that the aerobic threshold has been exceed. Special verbal and write explanations about the rating perceived are available for subjects (24) (See annex 3).

### 3.2.2. Current recommendations. (25)

The intensity of activity needed to improve physical conditioning varies among individuals and may be as low as 40% of VO2 max for 20 minutes, 3 times per week. However, lower intensity requires more dose of exercise than higher intensity in order to increase functional capacity. On the other hand, moderate-intensity exercise has a decreased likelihood of complications (as orthopedic injuries or higher dropout rates), compared to vigorous exercise.

Current physical activity guidelines recommend practical, regular, and moderate regimens for exercise. The standard exercise recommendation to promote and maintain health in adults aged 18 to 65 year need is a daily minimum of 30 minutes of moderate-intensity (such as brisk walking) physical activity, five to seven days per week or vigorous-intensity aerobic activity, like jogging, for a minimum of 20 min on three days each week [I(A)]. Also, combinations of moderate and vigorous intensity activity can be performed to meet this recommendation [IIa (B)]. In addition, every adult should perform activities that maintain or increase muscular strength. It is recommended that 8–10 exercises be performed on two or more nonconsecutive days each week using the major muscle groups. [IIa (A)]
Because of the dose-response relation between physical activity and health, persons who wish to further improve their personal fitness, reduce their risk for chronic diseases and disabilities or prevent unhealthy weight gain may benefit by exceeding the minimum recommended amounts of physical activity [I (A)]. For those unable to achieve this goal, instead of remaining sedentary, is preferable to do physical activity at lower intensity or shorter duration.

3.2.3. Exercise as part of metabolic syndrome therapy.

Current scientific evidence supports the role of exercise as an effective treatment strategy for the metabolic syndrome. Katzmarzyk et al. reported that 30.5% of the metabolic syndrome patients were no longer classified as having the syndrome after 20 weeks of supervised aerobic training. These patients experienced an improvement in many of the different thresholds for the ATP III classification. (26)

Moderate-intensity exercise, in moderate doses and in the absence of dietary changes, compared to vigorous exercise, may be more effective in improving metabolic syndrome, supporting the recommendation for adults to get 30 minutes of moderate intensity exercise on most days of the week and preferably every day. An exercise dose response effect has also been recognized, as an increased amount of exercise had greater and more widespread benefits. (27)

In the studies mentioned above, exercise was studied as the main intervention for improving the metabolic syndrome. However, normally this intervention is tested as part of the lifestyle interventions, jointly with diet prescriptions (28).

As it was explained before, weight is an important risk factor for metabolic syndrome development. There is enough evidence about the positive additive effects of diet and exercise interventions on body weight reduction. What is more, physical exercise and activity are also important for maintaining long-term weight loss and its beneficial in preserving lean body mass while being on a diet. (29)

When exercise is evaluated as a single treatment for weight loss, just many reductions in weight compared with no treatment (30). Probably, this fact is related to the difficulty in adhering to vigorous exercise regimens, as well as the fact that exercise training increase muscle mass resulting in less net weight loss.

However, exercise may be beneficial beyond its effect on weight loss by reduction in total body fat, abdominal obesity, visceral fat and insulin resistance (31). What is more, exercise promotes the lipolitic effect of catecholamines, that seem to be suppressed in obese individuals and people with hyperinsulinism.(32).

Removing abdominal adipose tissue with liposuction does not improve sensitivity or risk factors for coronary heart disease, suggesting that the negative energy balance induced by diet and exercise are necessary for achieving the metabolic benefits of weight loss. (33)
3.3. Natriuretic peptides.

The first measurements of circulating atrial natriuretic peptides (ANP) in humans were realized in early 1980s and since that date huge research has been done about the usefulness of this marker in many areas.

3.3.1. Biochemistry. (32,34,35)

There are three main types of natriuretic peptides: atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and C type natriuretic peptide (CNP). CNP is the most prevalent of the three peptides in the central nervous system but it is mainly produced by the vascular endothelium, acting as a paracrine factor by controlling the vascular tone. Urodilatin is an NH2 terminal-extended form of circulating ANP that is synthesized in the kidney acting as a paracrine factor that is not released to the systemic circulation. It participates in natriuresis regulation under physiologic conditions.

Each have a 17 amino acid ring structure in which some of the amino acid residues are conserved across the members of the family.

![Figure 2](image-url)  
*Figure 2. Extracted from article “Biochemistry of natriuretic peptides” (34). Mature forms of peptides belonging to the human atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP) families. Filled circles are amino acids, that are common to the three members of the human natriuretic peptide family. The mature forms shown are ANP, BNP-32 and CNP-22.*
Table 4. NP main characteristics. Based on data from (32,34,35)

<table>
<thead>
<tr>
<th></th>
<th>ANP</th>
<th>BNP *</th>
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<tbody>
<tr>
<td>Composition</td>
<td>28aa</td>
<td>32aa</td>
</tr>
<tr>
<td>Receptors **</td>
<td>NPR-A,NPR-C</td>
<td>NPR-A,NPR-C</td>
</tr>
<tr>
<td>Preprohormone</td>
<td>Pre-proANP (151 aa)</td>
<td>Prepro-BNP (134aa)</td>
</tr>
<tr>
<td>Prohormone</td>
<td>Pro-ANP (126 aa)</td>
<td>Pro-BNP (108aa)</td>
</tr>
<tr>
<td>Prohormone storage</td>
<td>Auricle granules (atrial myocytes)</td>
<td>None, directly released in ventricles as BNP hormone</td>
</tr>
<tr>
<td>Circulating peptides</td>
<td>NT-ProANP, ANP</td>
<td>NT-ProBNP, BNP</td>
</tr>
<tr>
<td>Biologically active</td>
<td>ANP</td>
<td>BNP</td>
</tr>
<tr>
<td>Plasmatic T ½</td>
<td>3´</td>
<td>21”</td>
</tr>
<tr>
<td>Synthesis place</td>
<td>Auricle</td>
<td>Ventricle</td>
</tr>
</tbody>
</table>

* Brain natriuretic peptide is synthesized as a 134-amino acid preprohormone, which is processed to a 108-amino acid propeptide (pro-BNP) by the cleavage of N-terminal 26 aa signal peptide. Upon secretion from the cardiomyocyte, pro-BNP is cleaved by corin and furin into equimolar amounts to the physiologically active BNP (32 amino acids) and a biologically inactive 76-amino acid fragment NT-pro-BNP. (See annex 5)

** NPR-A and NPR-B are guanylyl cyclase coupled transmembrane receptors, whereas NPR-C is a clearance receptor (G-proteins signal pathway). Both ANP and BNP receptors activate guanylate cyclase to generate cyclic guanosine monophosphate (cGMP) which results in vasodilatation. ANP and BNP both have more affinity for NPR-A. Meanwhile, CNP have a higher affinity for NPR-B. NPR-C is mainly present in adipose tissue and kidney and it has higher affinity for ANP. This fact can explain why BNP half-life is much longer compared with that of ANP. Natriuretic peptides are also cleared by neprelysin (neural endopeptidase), a metalloproteinase richly found in the kidney and lung. (32)

### 3.3.2. Control of natriuretic peptide release (35,36)

ANP and BNP are both continuously released from the heart. Atrial muscle mechanical stretch is well known to augment ANP and BNP release, although the precise mechanism is unknown. However, this mechanism is thought to be the increase in atrial dimension, rather than changes in atrial pressure.

ANP is released mainly from the atria, while BNP and NT-pro BNP are mainly products of ventricular myocytes and, unlike ANP, BNP resides primarily in ventricular muscle.

When CHF happens, ventricular BNP secretion increases drastically, indicating great ventricular stress and serious cardiac dysfunction. The same thing happens with ANP release in the auricle.
3.3.3. Current uses for Natriuretic peptides.

There is a large body of evidence supporting the use of the natriuretic peptides (both BNP and NT-proBNP) for the evaluation and management of patients with HF in a variety of clinical settings (see table 5).

Table 5. Current indications for natriuretic peptide measurements in HF. Modified from ACC/AHA guideline (37).

<table>
<thead>
<tr>
<th>Indication</th>
<th>ACC/AHA Recommendation Class-Level of Evidence</th>
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<tbody>
<tr>
<td>Diagnosis in patients with dyspnea (acute/ambulatory) *</td>
<td>I-A</td>
</tr>
<tr>
<td>Prognosis in patients with known HF (acute/ambulatory)**</td>
<td>I-A</td>
</tr>
<tr>
<td>Achieving guideline-directed medical therapy (ambulatory)</td>
<td>IIa-B</td>
</tr>
<tr>
<td>Natriuretic peptide-guided therapy for chronic HF</td>
<td>IIb-B</td>
</tr>
</tbody>
</table>

Class I: conditions for which there is evidence and/or general agreement that a given procedure or treatment is useful and effective; Class IIa: weight of evidence/opinion is in favor of usefulness/efficacy; Class IIb: usefulness/efficacy is less well established by evidence/opinion. Level of Evidence: A: recommendation on the basis of evidence from multiple randomized trials or meta-analyses; Level of Evidence: B: recommendation on the basis of evidence from a single randomized trial or nonrandomized studies.

* BNP threshold of 100 pg/ml have been proved to distinguish between a diagnosis of HF and other causes of dyspnea with a high degree of accuracy in emergency setting. Moreover, BNP <100 pg/ml suggest that such patients with acute dyspnea have a very low likelihood of having acute HF as an explanation for their symptoms (38). Similar data with NT-proBNP specifies the need of age-related thresholds: NT-proBNP >450 pg/ml for patients <50 years of age and NTproBNP >900 pg/ml for patients 50 years of age or older. (39)

Despite there are fixed thresholds for the diagnose of HF, the interpretation of these biomarkers need to be individualized in each context, taking into account the possibility of false negatives (obese patients tend to have lower levels of circulating peptide levels for a given degree of HF), other conditions that may elevate natriuretic peptide levels (e.g. aging and chronic kidney disease) and other therapies into account. Actually, euvolemic patients can have higher ranges (particularly, older women with lower lean mass). Analytical variability, between different laboratory-based testing, often difficult to harmonize diagnostic ranges. The measurement of any biomarker in isolation, without clinical context, will never be acceptable. Nevertheless, natriuretic peptide measurements are readily available and widely used, but still do not always provide clear-cut guidance regarding specific therapies. (35)
3.3.4. Effects of natriuretic peptides.

**Figure 3.** Target organs of the natriuretic peptide system. Extracted from (40). Renin–angiotensin–aldosterone system (RAAS); White adipose tissue (WAT); HSL hormone sensitive lipase.

**I. Cardiovascular effects of natriuretic peptides.**

Cardiac natriuretic peptides are released from the heart under physiological conditions and are involved in cardiovascular homeostasis, achieved by the action of those peptides in many organs:(35,36)

**A) Renal system:**
- Increase of glomerular filtration (by dilatation of afferent and constriction of efferent renal arterioles), which leads to enhanced natriuresis and diuresis.
- Inhibition of renin–angiotensin–aldosterone system (RAAS), release of renin from the kidney and aldosterone from the adrenal gland, which enhances natriuresis and reduces extracellular volume.

**B) Vascular system.** Reduction of vascular tone, decreasing systemic and peripheral vascular resistance.
- Natriuretic peptides suppress sympathetic nerve activity in the peripheral vasculature by suppressing catecholamine release from the nerve endings, and by the inhibition of the sympathetic outflow.
- Because these peptides lower the activation threshold of vagal afferents, reflex tachycardia and vasoconstriction that follow reduction in the preload are suppressed, causing a sustained decrease in mean arterial pressure.
II. Metabolic effects of natriuretic peptides.

Natriuretic peptides are mainly known for their role in cardiovascular homeostasis. However, during the last decade, a wide range of metabolic effects of these molecules have been discovered. This fact proves that NPs have also an active role in the defense against metabolic disease. (40)

A) Natriuretic peptide activated lipolysis in human adipocytes.

Figure 4. NP related effects in human adipocytes. Extracted from (40). BNP brain type natriuretic peptide; NPRA natriuretic peptide receptor A; NPRC natriuretic peptide receptor C; GCA guanylyl cyclase A; GTP guanosine triphosphate; cGMP cyclic guanosine monophosphate; PKG protein kinase G; ATP adenosine triphosphate; AC adenylyl cyclase; cAMP cyclic adenosine monophosphate; PKA protein kinase A; HSL hormone sensitive lipase; ATGL adipocyte triglyceride lipase; AMPK AMP-activated protein kinase; p38-MAPK mitogen-activated protein kinase; AKT protein kinase B.

Catecholamines induce lipolysis stimulating adrenergic β-receptors in adipocytes, and subsequent cAMP dependent activation of hormone sensitive lipase (HSL). Insulin causes the cAMP degradation, inhibiting lipolysis.

ANP and BNP stimulate NPRA inducing a cGMP dependent pathway, increasing intracellular cGMP levels, which activate cGMP dependent protein kinase G (PKG). These pathway ends with phosphorylation of perilipin-A, facilitating the binding of hormone sensitive lipase that turns into hydrolysis of triglycerides into free fatty acids and glycerol (41). Insulin may reduce NPRA expression and, at the same time enhance NPRC expression in white adipose tissue in rodents and humans, leading to lower thresholds of circulating NP. (42)
B) NP enhance lipid oxidation and mitochondrial respiration.

By the same cGMP dependent pathway, in skeletal muscle cells, NPs rise intracellular cGMP levels leading to expression of PGC1α downstream genes. Moreover free fatty acids from adipocyte lipolysis serve as additional ligands for the transcriptional regulator of mitochondrial biogenesis PPARδ. These mechanisms increase muscle mitochondrial content and mitochondrial respiration and lipid oxidation in skeletal muscle. This mechanism is supported by many studies in human cells and rodents, in vitro and in vivo (43), as well as by studies in human myotubes.

In white and ‘beige’ (brite) adipose tissue, NPs mediate similar effects by stimulating the same pathway that ends in the activation of the GK-I. This molecule is thought to activate the p38 mitogen protein kinase (p38 MAPK) (that stimulates UCP1 expression), as well as the AMP-activated protein kinase (AMPK) that increase transcriptional activity of PGC-1α. The result is mitochondrial biogenesis and white adipose tissue browning, increasing energy expenditure and thermogenesis.

The study by Bordicchia colleagues (1) proved that in genetically engineered mice lacking NPR-C, compared to wild-type mice, NP plasmatic levels were higher, fat mass was lower and genes characteristic of brown adipocytes were expressed at elevated levels, increasing thermogenesis and consumption of excess calories. The study also performed experiments in two separated human cell lines, with similarly results.
C) NP interactions with adipokines: enhancement of adiponectin production (an insulin sensitizing adipokine) (44) and a reduction in leptin release from human adipocytes.

D) Insulin secretion and glucose homeostasis. A number of studies suggest that NP might enhance insulin secretion from pancreas, increasing glucose disposition. (40)

E) Adipose tissue distribution. NPs may favorably affect adipose tissue distribution. Increased NP levels are associated with reductions in visceral adipose tissue (VAT) and in ectopic, intra-organ fat deposition such as intrahepatic lipid accumulation and, at the same time, increased lower body fat. This favorable profile may leads to less insulin resistance. (45)

3.3.5. Natriuretic peptides in obesity.

There are many factors that influence in the level of circulating NPs. Recently, researchers have demonstrated a consistent inverse relationship between obesity (defined as having a BMI of 30 or greater) and circulating BNP levels. Therefore, in obese patients, levels tend to be lower and the established diagnostic threshold of HF can lead to misdiagnose obese patients with acute dyspnea (36). The first study to describe the inverse relationship between obesity and BNP levels was a cross-sectional study. (46) Some cohort studies have confirmed the inverse relationship among BMI, BNP, and NT-proBNP levels. (47) Decreased NT-proBNP levels were also observed in obese patients with stable as well as acute CHF (48). Many potential causes have been studied:

- **Renal function.** has been reported to depress BNP levels in obese CHF patients because obesity is associated with higher glomerular filtration rates (49).

- **Adipose tissue expansion in obesity.** As it was mentioned before, NPs are cleared by neprelysin and NPR-C receptors, and both are expressed in adipose tissue. NPR-C is overexpressed in adipose tissue in obese patients while NPR-A expression is lower, which implicates higher degradation of NPs, facilitating weight gain by the multiple mechanism that were explained before (41). Moreover, hyperinsulinemia (common in obesity) induce NPR-C expression in monocytes (42) and human adypocytes, independently of circulating glucose concentrations. However, the Dallas Heart Study showed that obesity is also linked with lower levels of NT-proBNP, which is not cleared by the NPR-C (47).

In addition, neprelysin is expressed at increased levels in adipose tissue of obese people, leading to higher clearance. (50)

To sum up, the evidence tends to show that in obese patients a higher clearance may be one of the causes of lower NPs circulating thresholds.
3.3.6. Natriuretic peptides in insulin resistance and diabetes.

A potential connection between plasma glucose and insulin levels and the natriuretic peptide system has been observed in several studies. As it was explained before, insulin has been proved to up-regulate NPR-C in obese patients. What is more, in some studies, the inverse relation between NT-proBNP levels and visceral adipose mass, was attenuated after adjustment for HOMA-IR (homeostasis model assessment of insulin resistance), emphasizing the role for hyperinsulinemia in lower natriuretic peptide levels in obesity. (51)

On the other hand, many studies have suggest that NPs might be protective against DM2 and against insulin resistance (45).
4. JUSTIFICATION.

Since the 1980s, a huge amount of literature about natriuretic peptides has been produced. They are well known for their role in cardiovascular homeostasis by stimulating natriuresis, diuresis and vasodilatation. What is more, there is a large body of evidence supporting the use of NPs for the evaluation and management of patients with HF. (37)

However, during the last decade, a wide range of novel findings about natriuretic peptides proves that NPs have also an active role in the defense against metabolic disease. NPs have been found to enhance lipolysis in human adipocytes (41), to increase mitochondrial respiration and lipid oxidation in skeletal muscle (43) and to stimulate the browning of white adipose tissue, leading to the increase in thermogenesis and energy expenditure (1). To sum up, NPs prevent the accumulation of fatty acids and increase the basal metabolism. What is more, NPs may favorably affect adipose tissue distribution, reducing visceral adipose tissue distribution and increasing lower body fat, leading to less insulin resistance (45). Regarding to effects on insulin metabolism, NPs have been found to increase both insulin secretion from pancreas (52) and insulin sensitivity (through adiponectin production and other mechanisms) (44).

On the other hand, evidence has shown obesity and insulin resistance to be associated with lower levels of circulating natriuretic peptides. (46–48) Thus, people with this metabolic profile, as patients with metabolic syndrome, could be stuck in a vicious cycle in which a suppressed natriuretic peptide system leads to further impairment in the ability to resist fat accumulation. Moreover, in a population without manifest cardiovascular disease, as the metabolic syndrome, a decrease of cardiovascular protective hormones may increase long-term cardiovascular risk. In this situation, any measure that could help to increase circulation and action of NPs could help to break this cycle, improving the metabolic profile.

Fortunately, apart from the classical hemodynamic triggers of natriuretic peptide release, a number of novel stimuli have been described like cold exposure (1), weight loss (53) or exercise (54). These observations raise the possibility that the natriuretic peptide system could contribute to the favorable metabolic effects of lifestyle intervention measures as exercise. Regular endurance physical training have been shown to improve lipid-mobilizing effects of ANP in overweight subjects (55), as well as to improve many of the components of the metabolic syndrome (26,27,30). However, there isn’t any study about the exercise long-term effect in the concentration of NPs in patients with metabolic syndrome.

Therefore, it would be interesting to study if part of the beneficial effects of exercise training, in patients with metabolic syndrome, is due to NPs increase. Our study is an exploratory and hypothesis generating study. The main goal is to study if a 2 month moderate-intensity exercise intervention could lead to a significant increase in natriuretic peptide levels in patients with metabolic syndrome and to study if the benefits of this exercise intervention could be partially due to this increase.
We will also measure the improvement of the insulin resistance condition (measured by HOMA index). If the results were favorable to this association, NPs could provide a promising target for the treatment of metabolic syndrome and other metabolic disorders as obesity. Because of their effects on mitochondrial, lipid and glucose metabolism and on arterial blood pressure, these molecules could be a promising approach to simultaneously address cardiovascular and metabolic conditions. Despite their metabolic effects are shared by the adrenergic system, NP might avoid the deleterious effects of adrenergic activation on cardiovascular function, being almost ideally for the fight against cardiometabolic disease and reducing the risk of developing all the diseases that are related to metabolic syndrome and that represent a huge burden for the sanitary system (cardiovascular disease and type 2 diabetes mellitus).

5. HYPOTHESIS.
2-month moderate-intensity endurance exercise training, as a lifestyle modification intervention, may lead to an increase in NPs circulating levels in patients with metabolic syndrome and a sedentary lifestyle.

6. OBJECTIVES.

6.1. Main objective.
To study if 2 months of moderate-intensity endurance exercise training leads to an increase in NPs circulating levels (NT-ProBNP) in patients with metabolic syndrome and a sedentary lifestyle.

6.2. Secondary objectives.
- To study if our exercise program leads to an improvement of the metabolic syndrome components:
  - Abdominal obesity defined by waist circumference: ≥102 cm in men and ≥88 cm in women, except for patients from South Asia, Japan and China which appropriate threshold is ≥90 cm in men and ≥80 cm in women.
  - Altered fasting plasma glucose: ≥100mg/dL.
  - High blood pressure: ≥130/85.
  - Low serum high-density lipoproteins (HDL) cholesterol: <40mg/dL in men and <50mg/dL in women.
  - High serum triglycerides: ≥150mg/dL.
- To determine if the exercise intervention leads to a reduction in the insulin resistance condition (measured by HOMA index).
- To study the change in subjects physical limitations and cardiovascular fitness with our exercise intervention (VO2 max or maximal oxygen consumption, heart rate and relative perceived exertion).
7. METHODOLOGY.

7.1. Study design.
This study will be a controlled, randomized and single-center clinical trial, designed to evaluate the efficacy of a 2-month moderate-intensity endurance exercise training intervention in the improvement of natriuretic peptides levels and other altered parameters in patients with metabolic syndrome and a sedentary lifestyle. The patients will be randomly divided in two groups (1:1). The first group will go under an exercise training program and the second group will be the control group that will maintain their baseline sedentary lifestyle.

It will be an open study, but observer blind will be done. Therefore, participants and medical professionals that will perform the procedure will know the group assignment, but the staff who will make the monitoring at the end of the study and the statistical analyst who will analyze the results won’t have that information.

This study will be conducted in Hospital Santa Caterina, in the Sports Medicine Department and patient sampling will take place in the CAP of the 4 ABS of the city of Girona.

7.2. Study population.

7.2.1. Inclusion criteria.

- Participants with metabolic syndrome defined as the current criteria of the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (see annex 1).
- Elevated waist circumference (≥102 cm in men and ≥88 cm in women, except for patients from South Asia, Japan and China which appropriate threshold is ≥90 cm in men and ≥80 cm in women) (3).
- Age ≥18 years old and ≤65 years old.
- Sedentary lifestyle at baseline defined as reporting exercising less than 3 days per week for less than 20 minutes per day over the previous 6 months.
7.2.2. Exclusion criteria.

- Cardiovascular disease that contraindicates exercise practice: known history of cardiovascular event, unstable angina pectoris, decompensated heart failure, uncontrolled cardiac arrhythmias, exercise-induced ventricular tachycardia, high degree AV block, active endocarditis, myocarditis or pericarditis, moderate stenotic valvular heart disease, hypertrophic cardiomyopathy and ventricular or aortic aneurism.
- Uncontrolled hypertension (180/110 mm Hg)
- Aortic dissection
- High-intensity RT (80% to 100% of 1-RM) in patients with active proliferative retinopathy or moderate or worse non proliferative diabetic retinopathy.
- Uncontrolled hyperthyroidism with clinical manifestations as tachycardia or arrhythmia.
- Severe respiratory and liver failure or chronic renal failure with <60ml/min of glomerular filtration rate.
- Severe pulmonary hypertension (mean pulmonary arterial pressure 55 mm Hg)
- Recent pulmonary embolism or pulmonary infarction.
- Partially or poorly controlled asthma
- Impaired cognitive function leading to inability to cooperate (dementia, delirium, drug addiction…) or known eating disorder.
- Neurological conditions in which exercise may be contraindicated include the following: convulsive disorder not completely controlled by medication, history of intracranial bleeding within the last year, myopathies, myasthenia, ELA disease, advanced post-polio syndrome and syringomyelia.
- Cancer.
- Acute and chronic infections.
- Pregnancy.
- Musculoskeletal or orthopedic limitations or other physical disability that would preclude safe and adequate exercise performance.
7.3. Sample & Sampling methods.

7.3.1. Sample size.
To calculate the sample size for our principal variable (the increase of NPs circulating plasma levels), calculations were realized using GRANMO application. We accepted an alpha risk of 0.05 and a beta risk of 0.2, in a two-sided test, anticipating a drop-out rate of 15%.

A study group of 66 patients is needed and 33 subjects will be included in each group (randomization of 1:1), in order to recognize as statistically significant a minimum difference of 15% between both groups. The common standard deviation is assumed to be 20%.

7.3.2. Sampling methods.
As there is not an official register of patients with metabolic syndrome in Girona, we don’t have a sampling frame. Consequently, a non-probabilistic consecutive sampling method will be used in order to get patients that meet criteria for metabolic syndrome (as it was defined in the inclusion criteria). The patients will be recruited to the study from their general practitioner consultation. The CAPS who will be asked to participate in the recruitment are those in the 4 ABS of Girona:

- ABS 1: CAP Santa Clara.
- ABS 2: CAP Can Gibert del Pla.
- ABS 4: CAP Dr.Joan Vilaplana.

A meeting will be done with the general practitioners of these centers to explain the study and they will be given a copy of the protocol. Those physicians interested in participation in recruitment will be provided with the information sheet for the patient and the informed consent sheet. If a patient meets the metabolic syndrome criteria and the other inclusion criteria of the study and doesn’t meet exclusion criteria, the general practitioner will explain to them what the study consist on, the intervention characteristics and the possible side effects of the procedure. All this information will be explained in the information sheet (see annex 6). After being informed, if patients agree to participate in the study, the informed consent will be provided (see annex 7) and after signing it they will be referred to the Department of sports medicine in Santa Caterina, the reference center. There, they will undergo an exercise testing (explained in the procedures) and if there are no contraindications for the exercise intervention, they will be included to the study and randomly assigned to the different treatment groups.
7.3.3. Estimated time of recruitment.

According to recent studies (9), the prevalence of metabolic syndrome in Spain is 10.2%. Considering the population of Girona (http://www.idescat.cat/emex/?id=170792&lang=es), and the SIDIAP (http://www.sidiap.org/index.php/ca/base-de-dades/aspectes-generals) estimations of a 74% of patients going to the general practitioner at least once a year in the ABS participating in the study, we have enough prevalence to recruit our 66 patients in two months.

7.3.4. Randomization procedures.

Randomization process will be performed by the main investigator by simple randomization computer-generated, distributing the patients in the two different groups (control group or exercise group) by a 1:1 ratio.

Patients meeting inclusion criteria

↓

Information sheet and informed consent

↓

Exercise testing screening

↓

Randomization (1:1) to exercise or control group

7.3.5. Blinding.

Because of the characteristics of our intervention (exercise or no exercise) it is not possible to carry out a simple or a double blind clinical trial. Therefore, this study will be an open study. In order to reduce the bias of an open study, observer blind will be done. To sum up, although both participants and medical professionals that will perform the procedure will know the group assignment, neither the staff who will make the monitoring at the end of the study nor the statistical analyst who will analyze the results will have that information.

After randomization, all the patients will receive an identification number in order to protect their anonymity and for maintaining the blind to the statistician.
7.4. Procedures.

Before initiating an exercise program, a pre-exercise medical evaluation for exercise prescription must be performed. Care must be taken to ensure that apparently healthy individuals who are beginning an exercise program do not have any disease that contraindicates physical exercising. Therefore, before our study starts, all potential exercise participants will undergo a pre participation screening based on a revision of the medical history, a brief medical interview (see annex 5), a physical examination (including the anthropometric measurements and basal constants), basic blood analysis, and a resting standard 12-lead electrocardiogram (ECG) an standing ECG and blood pressure (in the sitting position) to determine vasoregulatory abnormalities and positional changes and an echocardiography. A detailed explanation of the testing procedure and the risks and possible complications is included in the information sheet that will be given by the general practitioner before the recruitment, as it was explained before (see annex 6). All the medications that can alter the test results should be recorded and taken into account. This medical evaluation will be repeated at the end of the intervention in order to study how the exercise intervention affects to the different variables in our study. The data collected will be included in the statistical analysis.

The testing procedure will be realized with a cycle ergometer that is calibrated in watts (W) and can be converted to oxygen uptake in millimeters per minute. METs are obtained by dividing VO2 millimeters per minute/body weight (Kg) x 3.5.

The cycling test would consist on an initial warm-up (low load) at 25 W followed by increases of 25W every 3 minutes until exhaustion. VO2max will be measured using an ergo-spirometer (MMC Horizon System: SensorMedics,Yorba Linda,California USA) as well as heart rate using a Polar Sports tester heart rate recorder. The rating of Relative Perceived Exertion (RPE) will be measured using the Borg Scale (see annex 3).

Subjects will be supine in the post exercise period and monitoring will continue until blood pressure, heart rate and ST segments are approximate to baseline values.

This procedure will be performed under the supervision of a physician who is appropriately trained to administer exercise test, to interpret data, to recognize and prevent adverse events, to stop the test if is necessary and to provide advanced cardiopulmonary resuscitation (RCP).
All screened subjects that meet protocol criteria and that are interested will be randomly assigned to one of the two groups of intervention:

A) Exercise group.

- The intervention duration will be 2 months.
- Participant will do exercise 3 times per week and each session will last 45 minutes. After the first month, duration will be increased to 60 minutes per session.
- The type of exercise will be endurance training and the approved modalities are treadmills, elliptical machines and cycle ergometers. Exercising at low intensity for 5 minutes before (warm-up) and after (cool-down) the training session will be performed in order to help stretch and warm up muscles and ligaments and to reduce the risk of injury and to prevent hypotension which may occur with the sudden cessation of exercise.
- The subjects will do exercise according to the prescribed target heart rate of 55-70%, corresponding to 40-60% VO2max of their peak of oxygen (measured by the cycle ergometer test at baseline) that is equivalent to a perceived exertion levels of 12-13 on Borg scale (somewhat hard) (see annex 3).
- Heart rate will be measured by a polar heart rate recorder and registered in a heart rate file.
- Each session will be supervised by a personal physical coach that will register the attendance of each work out and the number of minutes completed within the assigned heart rate range. The adherence will be calculated as the number of minutes completed in the assigned heart rate each week, divided by minutes prescribed. All this information will be registered in a training diary (including day to day activities) as well as additional physical activity performed by some participants. Food and calorie intake were not modified during the training protocol.

B) Control group.

Participants in this group will be told not to change their lifestyle during the trial. At randomization, they will be offered supervised physical training in the same conditions as the exercise group once the trial had been finished.

The exercise intervention will take place in the GEIEG Sant Narcis sportive complex. And the participants will be divided in three groups (11 patients per group), based on the subjects availability to the different schedules. All groups will be supervised by the same personal trainer.
7.5. Study variables and methods of measurement.

7.5.1. Independent variable.
The independent variable will be being allocated in the exercise group or in the control group. It will be measured as a nominal qualitative dichotomous variable: exercise yes/no. It will be expressed as the percentage of patients in each group.

7.5.2. Dependent variables

-Primary dependent variable.
The main outcome will be a minimum increase of 15% in NPs circulating plasma levels (NT-ProBNP), in the intervention group, in order to recognize as statistically significant the difference between both groups.

It will be defined as a continuous quantitative variable and it will be expressed as the mean ± standard deviation.

Fasting subjects will undergo phlebotomy in a supine position, typically between 8:00 and 9:00 AM. Venous blood will be collected in standard blood collection tubes containing EDTA. Samples will be maintained at 4°C for ≤ 4 hours and then centrifuged (1430g for 15 minutes) at 4°C. Plasma will be then removed and frozen at -70°C until assays will be performed.

NT-proBNP will be measured by an electrochemiluminescence immunoassay, the Elecsys proBNP platform (Roche Diagnostics). The CV for the NT-proBNP assay is 3.3% at a concentration of 282 ng/L and 3.0% at a concentration of 6012 ng/L. The measuring range with this test is 5 - 35,000 ng/L and the intermediate precision is 2.9 - 6.1 %. (56)
- **Secondary dependent variables**

  - The metabolic syndrome components (abdominal obesity defined by waist circumference, elevated fasting plasma glucose, elevated blood pressure, low serum high-density lipoproteins (HDL) cholesterol, high serum triglycerides). All of them are continuous quantitative variables and will be expressed as the mean ± standard deviation:

  - Abdominal obesity will be measured using waist circumference and it will be defined as: ≥102 cm in men and ≥88 cm in women, except for patients from South Asia, Japan and China which appropriate threshold is ≥90 cm in men and ≥80 cm in women. A measuring tape will be placed in a horizontal plane around abdomen at level of iliac crest, ensuring that tape is parallel to floor and does not compress the skin. Measurement will be made at the end of a normal expiration. Two recordings will be taken and the mean of the two measures will be used for the analysis.

  - High blood pressure will be defined as ≥130/85mmHg. Blood pressure will be measured in the supine position after 10 minutes of rest in a quiet room at a room temperature of 22ºC. Three recording will be made at 1-min intervals with automatic equipment and the mean of the last two measurements will be used for statistical analysis.

  - High serum triglycerides (≥150mg/dL) and low HDL (<40mg/dL in men and <50mg/dL in women) will be analyzed using nuclear magnetic resonance spectroscopy.

  - Altered fasting glucose (≥110mg/dL) will be measured by using an automated glucose analyzer (YSI).

  - Insulin resistance condition (measured by HOMA-IR index) is also a continuous quantitative variable and will be expressed as the mean ± standard deviation. The HOMA-IR index will be calculated as follows:

    \[
    \text{Fasting glucose (mg/dL) \times fasting insulin (U/mL)}/405.
    \]

    - Insulin will be measured by using a radioimmunoassay kit.

  - The change in subjects physical limitations and cardiovascular fitness will be measured by different types of variables that measure relative intensity of physical exercise:

    - VO2 max or maximal oxygen consumption: is a continuous quantitative variable and it will be expressed as a percentage. It will be measured by an ergo-spirometer during the exercise testing, as it was explained before.

    - Heart rate: is a continuous quantitative variable and it will be expressed as a percentage. It will be measured using a Polar Sports tester heart rate recorder.

    - Relative perceived exertion: is a continuous quantitative variable and it will be expressed as the mean ± standard deviation. It will be measured using the Borg scale (see annex 3).
7.5.3. Covariables.

To avoid confusion in the study results, we will collect some data from the patient’s clinical history and from the exercise testing medical evaluation, which can act as confusion factors and modify the interpretation of results:

- Age: is a discrete quantitative variable, expressed in years.
- Gender: is a dichotomous nominal qualitative variable: male or female.
- Ethnicity: is a dichotomous nominal qualitative variable: Caucasian or others.
- Smoking: is a dichotomous nominal qualitative variable that will be expressed as: yes/no.
- BMI will be calculated as: body mass (Kg) divided by the square of the body height (metres), and it will be expressed in units of kg/m². It is a continuous quantitative variable. Weight and height will be measured during the physical examination at baseline. Weight will be determined using a mechanical scale while wearing underwear and no shoes.
- Specific treatments: antihypertensive medication use and any treatment for elevated blood glucose or dyslipemia. All are dichotomous nominal qualitative variables that will be expressed as: yes or no.
- Blood pressure: it is a continuous quantitative variable and it will be expressed as the mean ± standard deviation.
- Diabetes diagnose, expressed as a dichotomous nominal qualitative variable: yes or no.
- Left ventricular mass (gr), end diastolic volume (mL) and ejection fraction (%) will be collected during the echocardiography procedure at baseline. This procedure will be carried on by one only cardiologist in order to decrease interindividual variability. All are continuous quantitative variables and will be expressed as the mean ± standard deviation.
7.6. Data collection.

Before the recruitment starts all the general practitioners that want to do the recruitment will be instructed in how to identify the potential individuals that meet criteria for the metabolic syndrome, in order to guarantee the homogeneity in the recruitment.

As it was explained before, the patients will be referred by their healthcare provider to the hospital of reference, Santa Caterina Hospital. There, all clinical measurements, blood test, questionnaires and the pre exercise screening will be carried out in the Sports of Medicine center at baseline and after the 2-month training intervention. During this medical evaluation, the individual components of the metabolic syndrome will be assessed.

Blood samples will be collected by a nurse in Hospital Santa Caterina after a 10-min recumbence between 8:00 and 10:00 AM, after an overnight fast and abstinence of smoking. What is more participants will be told to abstain exercise training for 4 days before blood samples in order to avoid an skew in the results because of the acute effect of exercising. The components will be analyzed batch wise from frozen samples at the end of the trial in the laboratory department of Hospital Santa Caterina.

To sum up, data collection will be performed by the general practitioner in recruitment, during the exercise test and medical evaluation (pre and post intervention) by the doctors of Sports of Medicine Department, by the nurse that will obtain blood samples and the analysis department and during the intervention by the personal trainer. Before the data collection starts, this stuff will be trained in order to do it properly.
8. STATISTICAL ANALYSIS.

Firstly, a table will be done with the characteristics of the study population with the percentage for categorical variables and the mean ± standard deviation for continuous variables of each variable that we have registered. Then, in order to know the distribution of the study population according to their allocation to treatment or control group a bivariate analysis will be performed using the Student test to compare continuous variables between the groups, and the Chi² test for categorical variables and the proportions will be presented by absolute numbers and percentages. This analysis is important to see if randomization is actually uniformly distributed in each population group. If the two groups aren’t uniformly distributed, the results of the study could be affected by this randomization error. Therefore, all the covariables that could skew the main association we want to analyze, for being a randomization error, would be included in the multivariable analysis (explained below). However, when we use randomization for dividing patients in two groups, covariables that could skew the main association are usually equally distributed. Therefore, in this studies multivariate analysis is only necessary if randomization process fail in the equally distribution.

- **Univariate analysis:** The results will be expressed as percentages for categorical variables and as the mean ± standard deviation for continuous variables, assuming a normal distribution. If is not possible to assume a normal distribution, median (using quartiles or percentiles) will be estimated.

- **Bivariate analysis:** Then, a second bivariate analysis will be performed. Because the independent variable is categorical with two components (control group or treatment group), we will use a Student test to compare continuous variables between the two groups and the Chi² test for categorical variables. The primary dependent variable is the natriuretic peptide concentration in plasma.

A confidence interval of 95% will be assumed and we will consider p value <0.05 to consider that there is a significant difference.

- **Multivariate analysis:** As it was explained before, when we use randomization for dividing patients in two groups, covariables that could skew the main association are usually equally distributed. Therefore, in this studies, multivariate analysis is only necessary if randomization process fail in the equally distribution. If that happen, a multivariate analysis would be performed in order to add all the covariables that could skew the main association we want to analyze, for being a randomization error. Considering that our primary dependent variable is a continuous quantitative variable, we would need to do a general linear model (GLM) in order to associate the principal variables adjusting to the covariables that could represent a cofounding factor.

Data analysis will be carried out using Statistical Package for the Social Sciences (SPSS Windows) and management and recording of data will be performed using Microsoft Excel.
9. STUDY LIMITATIONS.

Our study has some limitations that should be taking into consideration. The most important ones are explained below:

- Our study is a single-center study. The main limitation is that they have limited external validity and they are not necessarily generalizable to a broader population. On the other hand, there are many advantages comparing to a multi-center study: it is logistically easier, cheaper, they do not require prolonged negotiations on the study protocol, they simplify data collection, there is not risk of differences in treatment application between different centers and they typically deal with a less heterogeneous population, thereby diminishing confounding. As our study is kind of an exploratory study, a single-center design can be acceptable. If our study results seem to be significant, it could allow larger, multicenter studies to be planned appropriately and powered.

- As we don’t have a sampling frame, a non-probabilistic consecutive sampling method will be used in order to get patients that meet criteria for metabolic syndrome. This method is not randomized, so the individuals of the population don’t have the same likelihood to be recruited. What is more, we can have a selection bias called “the Healthy worker bias”. The patients that are more concern about their condition will be the ones to visit the general practitioner the most and they also will be the ones to accept joining the study. Another problem of the enrollment is that it will be done by different general practitioners of different centers. In order to unify the inclusion criteria, a meeting will be done with the general practitioners of these centers to explain the study and how they should classify a patient as having the metabolic syndrome.

- There is not clinical evidence about the effect of 2-month exercise training in the NP circulating levels. Therefore, to calculate sample size, we based the estimation of the expected increase rate on the increase that has been shown in clinical trials studying the combined effect of diet and exercise. The sample size estimated is enough for the differences we expected to find.

- Because of the contraindications of doing sport, we have a big list of exclusion criteria. This could lead to skew the selection of our patients. Therefore the study population will be more homogeny but we could have problems in the recruitment and with the results extrapolation.

- Because of the characteristics of our intervention (exercise or no exercise) it is not possible to carry out a simple or a double blind clinical trial. Therefore, this study will be an open study. In order to reduce the bias of an open study, observer blind will be done. Therefore, neither the staff who will make the monitoring at the end of the study nor the statistical analyst who will analyze the results will know the group assignment. What is more, the main dependent variable of the study is very objective. Therefore, the influence of a detection bias is unlikely to happen.
- We don’t know if a 2-month exercise intervention will be enough to find significant differences between both groups. In order to solve that problem we have increase the exercise dose recommended as the minimal (from 30 minutes to 45 and 60 during the last month), but not the intensity because we don’t want to compromise our participants health. On the other hand, the study is not too long for having a high dropout rate, comparing to other exercising clinical trials that have been performed before. Therefore, adherence and compliance will be reasonable good making the final analysis more reliable.

- In our study no control on dietary factors has been done.

Confounding variables that might skew the relationship of our main variables of study have been controlled by randomization and by performing a multivariate analysis (if randomization would be ineffective).

10. ETHICAL AND LEGAL CONSIDERATIONS.

This trial is designed in accordance with the medical ethics requirements defined on the World Medical Association Declaration of Helsinki for Ethical Principles for Medical Research Involving Human Subjects. June 1964. Last revision, October 2013.

Before carrying out our study, the research protocol will be presented to the Clinical Research Ethics Committee (CEIC) of Hospital Universitari Santa Caterina of Girona, in order to be evaluated. Modifications of the protocol will be done in case the CEIC consider it necessary. If it is accepted, we will ask for permission to perform it to the direction of the center.

As it is now recommended, the trial will be submitted to ClinicalTrials.gov (https://clinicaltrials.gov/) and it will be registered with an International Standard Randomized Controlled Trial Number.

Since we will not depart from a previously constructed database, we will need the acquiescence of the patients to participate in the study. All the potential participants will be informed of the purpose of the research in the general practitioner consultation where they will be given an information shit (see annex) with all the necessary information and an informed consent (see annex). Both documents will be written with comprehensive language to ensure a proper informed decision. They will only be part of the study once the informed consent has been signed. Patients will be free to leave the study if they want to.

The medical record information, information from the clinical history, names and surnames will be confidential, remaining anonymous when publishing results, according to “the Ley Orgánica 15/1999, del 13 de Diciembre, de Protección de Datos de Carácter Personal.”
In addition, we will take into consideration the Spanish Organic Law 14/2007, del 3 de Julio, de Investigación Biomédica, that regulates biomedical investigation involving humans and the invasive procedure if the CEIC consider that any of our procedure can be consider as invasive according to the official definition written in this law: “Procedimiento invasivo: toda intervención realizada con fines de investigación que implique un riesgo físico o psíquico para el sujeto afectado”.

As our intervention is related to exercise prescription many laws related to exercise prescription and practice will be followed:

- Medical Specialty of physical education and sport: Royal Decree 127/1984, of the 11 of January, that regulates the medical specialist formation and the obtaining of the specialist title.
- Training and accreditation of the sanitary staff in basic and advanced cardiopulmonary resuscitation (CPR). European Resuscitation Council certificate, 2006.

In our study, the control group is not being intervened. In order to respect the ethical principle of non-maleficence, we will just include in the study people with a previous sedentary lifestyle. Moreover, the study duration will be short and at the end of the study, the control group will be offered to do the same intervention that was performed by the exercise group during the study, in order to respect the principle of beneficence. However, in the case of exercise, it is not ethical to prevent those assigned to the control group from engaging in exercise (drop-ins).

The investigators of this project declare that there are no conflicts of interest, and that they do not receive any economic compensation to collaborate in the study.

11. IMPACT OF THE STUDY ON THE HEALTH SYSTEM.

Since the 1980s, a huge amount of literature about natriuretic peptides has been produced. They are well known for their role in cardiovascular homeostasis by stimulating natriuresis, diuresis and vasodilatation. What is more, there is a large body of evidence supporting the use of NPs for the evaluation and management of patients with HF. Moreover, during the last decade, a wide range of novel findings about natriuretic peptides proves that NPs have also an active role in the defense against metabolic disease. NPs have been found to prevent the accumulation of fatty acids and to increase the basal metabolism through many mechanisms: enhancing lipolysis in human adipocytes, increasing mitochondrial respiration and lipid oxidation in skeletal muscle and stimulating the browning of white adipose tissue, leading to the increase in thermogenesis and energy expenditure.
What is more, NPs may favorably affect adipose tissue distribution, reducing visceral adipose tissue distribution and increasing lower body fat, leading to less insulin resistance. Regarding to effects on insulin metabolism, NPs have been found to increase both insulin secretion from pancreas and insulin sensitivity (through adiponectin production and other mechanisms).

On the other hand, evidence has shown obesity and insulin resistance to be associated with lower levels of circulating natriuretic peptides. Thus, people with this metabolic profile, as patients with metabolic syndrome, could be stuck in a vicious cycle in which a suppressed natriuretic peptide system leads to further impairment in the ability to resist fat accumulation. Moreover, in a population without manifest cardiovascular disease, as the metabolic syndrome, a decrease of cardiovascular protective hormones may increase long-term cardiovascular risk. In this situation, any measure that could help to increase circulation and action of NPs could help to break this cycle, improving the metabolic profile.

Metabolic syndrome is a cluster of many risk factors for type 2 diabetes mellitus and cardiovascular disease and its prevalence is persistently increasing. Abdominal obesity, normally measured by waist circumference, is a strong predictor of the metabolic syndrome. Moreover, abdominal obesity correlates strongly with insulin resistance, especially visceral type. As we explained before, these two conditions have been related to lower circulating PNs, which leads to increase the long-term cardiovascular risk and also to aggravate the obesity and insulin resistance conditions.

Fortunately, apart from the classical hemodynamic triggers of natriuretic peptide release, a number of novel stimuli have been described like cold exposure, weight loss or exercise, suggesting the possibility that the natriuretic peptide system could contribute to the favorable metabolic effects of lifestyle intervention measures as exercise. Therefore, it would be interesting to study if part of the beneficial effects of exercise training, in patients with metabolic syndrome, is due to NPs increase and this is the main goal of our study. If the results were favorable to this association, NPs could provide a promising target for the treatment of metabolic syndrome and other metabolic disorders as obesity. Because of their effects on mitochondrial, lipid and glucose metabolism and on arterial blood pressure, these molecules could be a promising approach to simultaneously address cardiovascular and metabolic conditions. Despite their metabolic effects are shared by the adrenergic system, NP might avoid the deleterious effects of adrenergic activation on cardiovascular function, being almost ideally for the fight against cardiometabolic disease and reducing the risk of developing all the diseases that are related to metabolic syndrome and that represent a huge burden for the sanitary system (cardiovascular disease and type 2 diabetes mellitus).
12. WORK PLAN

Personnel involved in the research team will be composed by the principal researchers (PR) (doctors from the Sports Medicine department of Hospital Santa Caterina of Girona) and the collaborating researchers (CR): general practitioners from the CAPs participating in the recruitment, a cardiologist for doing the echocardiography, a nurse for blood sampling collection, a doctor from the analysis Department, a personal trainer and a statistical specialist.

The study will take an overall of 16 months. The organization of the study will be divided in 5 stages, explained below:

**Stage 1: Coordination phase and development of theoretical framework (4 months).** It will involve the investigators and collaborators.

- Protocol elaboration (PR): formulation of objectives and study variables definition in order to answer the main formulated hypothesis, involving literature search. The methodology of the study will be established.

- Recruitment of collaborating researchers (PR): the PR will explain the protocol to the general practitioners of the 4 ABS of Girona and they will be asked to join the recruitment process. Those doctors who are interested in the project will be joined and formed in how to do the recruitment in order to unify the process. Staff will be assigned for the different tasks. To guarantee the professionalism of the participating members we will ask for certain skills and experience regarding the techniques we will be applying and personnel training will take place.

- Organizational meeting: for the elaboration of a chronogram, after mutual agreement between all the personnel involved in the research team.

- Evaluation of the protocol and authorizations by the CEIC of the center Hospital Santa Caterina.

**Stage 2: Field research (4 months).** It will involve the investigators and collaborators.

- Recruitment of the sample and group assignment (2 months). The recruitment of the patients will be performed by the general practitioner in the different centers of the 4 ABS of Girona, in the period of two months. If a patient meets the inclusion criteria of the study, and don’t meet exclusion criteria, they will be given the information sheet (see annex 6) and after being informed, if patients agree to participate and they sign the informed consent (see annex 7), they will be referred to the Sports of Medicine Department in Santa Caterina, the reference center. There, they will undergo the exercise testing and if there are no contraindications for the exercise intervention, they will be included in the study and randomly assigned to the different treatment groups.
Intervention (2 months). Each group will receive their treatment according to the medical instructions given. The intervention duration will be 2 months. The personal trainer will carry out the intervention. Training and accreditation of the personal trainer in basic and advanced cardiopulmonary resuscitation (CPR) will be required. An INEFC degree accreditation will be also required.

Data collection will be performed by the general practitioner in recruitment, during the exercise test and medical evaluation (pre and post intervention) by the doctors of Sports of Medicine Department and analysis department, by the nurse who will do the blood sampling and during the intervention by the personal trainer. Before the data collection starts, this stuff will be trained in order to do it properly.

Stage 3: Processing data base and statistical analysis (2 months). It will involve the statistician and the investigators. Data collected will be entered in the database at the end of the study and it will be analyzed by the statistical analyst accordingly as specified in statistical analysis paragraph.

Stage 4: Interpretation of the results (2 months). The principal investigators will be the only involved. An interpretation, discussion and conclusion of the outcomes will be performed.

Stage 5: Publication of the results (4 months). The principal investigators will be the only involved. With all the results, they will elaborate the final report, and they will send it to different medical journals for their publication.
## 13. CHRONOGRAM

<table>
<thead>
<tr>
<th>Stage 1: Coordination and development of theoretical framework</th>
<th>2017</th>
<th>2018</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protocol elaboration</td>
<td>F M A M J J A S O N D J F M A M</td>
<td></td>
</tr>
<tr>
<td>Organizational meeting and elaboration of a chronogram</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evaluation and authorizations</td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Stage 2: Field research</th>
<th>2017</th>
<th>2018</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recruitment and group assignment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intervention</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Data collection</td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Stage 3: Processing database and statistical analysis</th>
<th>2017</th>
<th>2018</th>
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</thead>
<tbody>
<tr>
<td>Statistical analysis</td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Stage 4: Interpretation of the results</th>
<th>2017</th>
<th>2018</th>
</tr>
</thead>
<tbody>
<tr>
<td>Results interpretations</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stage 5: Publication of the results</th>
<th>2017</th>
<th>2018</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final report elaboration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Publication and dissemination</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## 14. BUDGET

<table>
<thead>
<tr>
<th>EXPENSES</th>
<th>QUANTITY</th>
<th>COSTS</th>
<th>TOTAL COST</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Staff costs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Statistical consulting</td>
<td>30h</td>
<td>30€/h</td>
<td>900,00€</td>
</tr>
<tr>
<td>Personal trainer *</td>
<td>72h</td>
<td>60€/h</td>
<td>4,320,00€</td>
</tr>
<tr>
<td><strong>Materials and services</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medical- sportive evaluation</td>
<td>66 (x2)</td>
<td>45€</td>
<td>5,940,00€</td>
</tr>
<tr>
<td>PN analysis</td>
<td>66 (x2)</td>
<td>31€</td>
<td>4,092,00€</td>
</tr>
<tr>
<td>Echocardiogram</td>
<td>66 (x2)</td>
<td>50€</td>
<td>6,600,00€</td>
</tr>
<tr>
<td>Polar heart rate monitor</td>
<td>11</td>
<td>35€</td>
<td>455,00€</td>
</tr>
<tr>
<td>Printing</td>
<td>66(x3)</td>
<td>0,04€</td>
<td>7,92€</td>
</tr>
<tr>
<td>Informed consent sheet</td>
<td>66</td>
<td>0,04€</td>
<td>2,64€</td>
</tr>
<tr>
<td><strong>Publication and dissemination</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Publication</td>
<td></td>
<td>1.500€</td>
<td>1.500,00€</td>
</tr>
<tr>
<td>Presentation to the SEEN</td>
<td>Inscription</td>
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<td>500€</td>
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<tr>
<td>Travelling</td>
<td></td>
<td>75€</td>
<td>150,00€</td>
</tr>
<tr>
<td>National Professional Congress</td>
<td>Accommodation and allowance</td>
<td>2</td>
<td>100€</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td></td>
<td>25,167,56 €</td>
</tr>
</tbody>
</table>

* The personal trainer will be needed 3h/day, 3days/week during 8 weeks. The patients in the intervention group (33 patients) will be divided in 3 groups of 11 patients and they will need 11 polar heart rate monitors.

** All the material devices are calculated for the 66 patients that will be tested pre and post intervention.
15. REFERENCES


# ANNEX 1: METABOLIC SYNDROME CLASSIFICATION.

TABLE 1. Previous Criteria Proposed for Clinical Diagnosis of Metabolic Syndrome. Modified from: (3)

<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Required</strong></td>
<td>One or more:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1. Insulin resistance (HOMA)</td>
<td>Insulin resistance or</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2. Fasting Glucose ≥110</td>
<td>fasting hyperinsulinemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>mg/dL</td>
<td>in top 25 percent</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3. 2-hour glucose ≥140 mg/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Other Required</strong></td>
<td>And ≥2 of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1. Fasting Glucose ≥110 mg/dL</td>
<td>Elevated fasting glucose</td>
<td></td>
<td>Elevated fasting glucose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. 2-hour glucose ≥140 mg/dL</td>
<td>(≥100 mg/dL) Or drug</td>
<td></td>
<td>≥100 mg/dL or diagnosed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>treatment for elevated blood</td>
<td></td>
<td>diabetes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HDL Cholesterol</strong></td>
<td>Men: &lt;35 mg/dL</td>
<td>Men: &lt;40 mg/dL</td>
<td>Men: &lt;40 mg/dL</td>
<td></td>
<td>Men: &lt;40 mg/dL</td>
</tr>
<tr>
<td></td>
<td>Women: &lt;40 mg/dL</td>
<td>Women: &lt;50 mg/dL</td>
<td>Women: &lt;50 mg/dL</td>
<td></td>
<td>Women: &lt;50 mg/dL</td>
</tr>
<tr>
<td><strong>Triglycerides</strong></td>
<td>≥150 mg/dL</td>
<td>≥180 mg/dL or drug treatment</td>
<td>≥150 mg/dL</td>
<td></td>
<td>≥150 mg/dL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>for dyslipemia</td>
<td></td>
<td></td>
<td>or elevate drug treatment</td>
</tr>
<tr>
<td><strong>Obesity</strong></td>
<td>One or more:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1. Waist/hip ratio:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Men: &gt;0.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Women: &gt;0.85</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2. BMI ≥30 kg/m²</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>High blood pressure or hypertension</strong></td>
<td>≥ 140/90 mmHg or drug treatment for hypertension</td>
<td>≥140/90 mmHg or drug treatment for hypertension</td>
<td>≥130/85 mmHg</td>
<td>≥130/85 mmHg</td>
<td>≥130/85 mmHg or drug treatment for hypertension</td>
</tr>
<tr>
<td><strong>Microalbuminuria</strong></td>
<td>≥20 mg/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ANNEX 2: Classification of Physical Activity Intensity (Endurance activity).

Table 2. Classification of Physical Activity Intensity. Extracted from (57).

<table>
<thead>
<tr>
<th>Intensity</th>
<th>Relative Intensity</th>
<th>Absolute Intensity in Healthy Adults (Age), METS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Relative Intensity</td>
<td>Young (20–30)</td>
</tr>
<tr>
<td></td>
<td>V̇O₂ peak, %</td>
<td>(20–30)</td>
</tr>
<tr>
<td>Very Light</td>
<td>&lt;20</td>
<td>&lt;2.4</td>
</tr>
<tr>
<td>Light</td>
<td>20–39</td>
<td>2.4–4.7</td>
</tr>
<tr>
<td>Moderate</td>
<td>40–59</td>
<td>4.8–7.1</td>
</tr>
<tr>
<td>Hard</td>
<td>60–84</td>
<td>7.2–13.1</td>
</tr>
<tr>
<td>Very hard</td>
<td>≥85</td>
<td>≥10.2</td>
</tr>
<tr>
<td>Maximum‡</td>
<td>100</td>
<td>12.0</td>
</tr>
</tbody>
</table>

*Based on 8 to 12 repetitions for persons <50 to 60 years old and 10 to 15 repetitions for persons aged ≥50–60 years.
†Borg rating of Relative Perceived Exertion (RPE), 6–20 scale.
ANNEX 3

Table 3. Borg Scale for Rating Perceived Exertion. Extracted from (24)

<table>
<thead>
<tr>
<th>20-Grade Scale</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Very, very light</td>
</tr>
<tr>
<td>7</td>
<td>Very light</td>
</tr>
<tr>
<td>8</td>
<td>Fairly light</td>
</tr>
<tr>
<td>9</td>
<td>Somewhat hard</td>
</tr>
<tr>
<td>10</td>
<td>Hard</td>
</tr>
<tr>
<td>11</td>
<td>Very hard</td>
</tr>
<tr>
<td>12</td>
<td>Very, very hard</td>
</tr>
</tbody>
</table>
Figure 3: Processing of BNP. Image extracted from the article McCullough PA, Omland T, Maisel AS. B-type natriuretic peptides: adiagnostic breakthrough for clinicians. Rev Cardiovasc Med. 2003.
ANNEX 5: Clinical interview questionnaire

**IASSport**

**QÜESTIONARI DE SALUT MÈDICO-ESPORTIU**

1) Hi ha alguna malaltia important a la família més directa? Problemes cardíacs, respiratoris,.....?

2) Heu tingut alguna malaltia mèdica important? Quan?

3) Us han intervingut quirúrgicament d’alguna cosa? Quan?

4) Heu tingut alguna lesió esportiva important? Quan?

5) Teniu algun tipus d’al·lèrgia? A què?

6) Quantes hores fem d’esport a la setmana? Des de quan fas esport?

El lot assignat declara que totes les dades exposades anteriorment són veridiques.

Nom i cognoms: ........................................................................

Data: ..............................................
ANNEX 6: INFORMATION SHEET
(Catalan and English versions will be also available)

HOJA INFORMATIVA PARA EL PACIENTE

Los péptidos natriuréticos como posible mediador de los efectos positivos del ejercicio físico en pacientes con síndrome metabólico: un ensayo clínico controlado y randomizado.

Por favor, lea la siguiente información con atención y antes de tomar la decisión de participar o no en el estudio, es imprescindible que lea y entienda la hoja informativa. Ante cualquier duda, pregunte al doctor que le invita a participar en el estudio.

Le informamos de se va a llevar a cabo un ensayo clínico con el objetivo de promover el ejercicio físico como tratamiento del síndrome metabólico en pacientes con un estilo de vida sedentario, con el objetivo de medir su eficacia en la mejora de los factores de riesgo de este síndrome y el estudio de los mecanismos por los cuales acontece esta mejora.

Como potencial participante le informamos de que este estudio ha sido aprobado por el Comité de Ética e Investigación Clínica del centro responsable del estudio, el Hospital Universitario Santa Caterina.

Es un estudio que tendrá lugar en un único centro. El equipo de investigadores clínicos del Servicio de Medicina del Deporte del Hospital Universitario Santa Caterina, junto al equipo de médicos de medicina de familia de las cuatro áreas básicas de Gerona, propone la realización del estudio mencionado, basado en observaciones propias y en trabajos científicos de investigación médica.

Esperamos incluir al menos 66 pacientes con síndrome metabólico y un estilo de vida sedentario, entre 18 y 65 años.

INTRODUCCIÓN

El síndrome metabólico es un conjunto de factores de riesgo cardiovascular, para el desarrollo de Diabetes tipo 2 y otros trastornos asociados. Estos factores incluyen dislipemia (triglicéridos altos en sangre y HDL bajo), presión arterial elevada, glucosa plasmática elevada en ayunas y obesidad abdominal. Los estudios a nivel estatal estiman una prevalencia de cerca del 10,2%. Por lo tanto, la identificación de los pacientes con dicho síndrome y la modificación del estilo de vida junto a otros tratamientos complementarios, puede reducir dichos factores de riesgo y, también el riesgo de desarrollar todas las enfermedades asociadas. La base de un estilo de vida saludable es mantener una dieta equilibrada y realizar ejercicio en las dosis e intensidades recomendadas actualmente.

Estas dos intervenciones han demostrado eficacia en la mejora de los parámetros del síndrome metabólico.
OBJETIVO

El objetivo de este estudio es observar si el ejercicio físico como intervención de modificación del estilo de vida mejora los parámetros del síndrome metabólico en pacientes con un estilo de vida sedentario y estudiar cuáles son los mediadores de esta mejora (péptidos natriuréticos) con el objetivo de generar nuevas dianas terapéuticas que nos permitan disminuir la incidencia de los factores de riesgo de éste síndrome y así prevenir la aparición de enfermedades asociadas.

PROCEDIMIENTOS

Previamente a la inclusión en el programa de ejercicio, todos los pacientes serán sometidos a una revisión médico-deportiva para descartar cualquier patología que pueda presentar el paciente y suponga una contraindicación para la realización del programa de ejercicio. Ésta consistirá en una revisión de la historia del paciente, una anamnesis, una exploración física y medición de las medidas antropométricas y constantes básicas, una analítica básica, una prueba de esfuerzo en un cicloergómetro, un ECG basal y durante el esfuerzo y un ecocardiograma. La prueba de esfuerzo determinará la respuesta cardiovascular al ejercicio físico, con el fin de ajustar la intervención a las limitaciones del individuo y disminuir así los riesgos que ésta conlleva. La prueba se realizará en un cicloergómetro, con aumentos graduales del esfuerzo hasta la aparición de síntomas como fatiga, dificultad para respirar, o molestias en el pecho. Si alguno de estos síntomas aparece indicar la necesidad de cesar el esfuerzo. Algunos cambios que pueden aparecer durante la prueba son: alteraciones en la presión o frecuencia cardíaca (rápido, lento o inefectivo), sensación de mareo y anomalías en el ECG reflejando el esfuerzo cardíaco. También existe un riesgo mínimo de infarto cardíaco y muerte súbita (tasas de 0-5 por cada 100.000 pruebas de esfuerzo). Estas pruebas se realizan bajo vigilancia médica constante por un médico formado en Resucitacion Cardiopulmonar (RCP) avanzada.

Los pacientes aptos para la realización del programa de ejercicio físico serán aleatorizados en dos grupos: el grupo de intervención, que realizará el programa de ejercicio físico durante el período de dos meses de duración del ensayo y el grupo control, que no modificará su estilo de vida sedentario hasta que concluya el ensayo. Entonces, se ofrecerá a los sujetos del grupo control la posibilidad de realizar la misma intervención que realizó el grupo intervenido durante el ensayo. Dicha intervención consistirá en la realización de un programa de ejercicio diseñado por un entrenador personal, que supervisará la asistencia a las sesiones y la adherencia al programa. Estos datos serán registrados en un diario de actividades. Se realizarán 3 sesiones por semana (lunes, miércoles y jueves) de una duración de 45 minutos durante el primer mes y 60 minutos durante el segundo mes. El grupo intervenido se dividirá en 3 grupos (11 pacientes por grupo), según la disponibilidad de los participantes a los distintos horarios ofrecidos y todos los grupos serán supervisados por el mismo entrenador personal. La intensidad del ejercicio a realizar será moderada. Las modalidades aprobadas son: cintas de correr, bicicletas elípticas y bicicletas estáticas. Las sesiones se llevarán a cabo en el Complejo Deportivo GEiEG Sant Narcís (https://www.fitnessgeieg.com/gimns-sant-narcs).
La medición de las variables de interés se realizará al inicio y al final del estudio. Nuestro objetivo es descubrir diferencias significativas entre los dos grupos para saber si el ejercicio supone o no una mejora de los parámetros a estudiar y si esta mejora está parcialmente mediada por el aumento de los péptidos natriuréticos.

**POSIBLES BENEFICIOS**

Tanto el grupo de intervención, como el grupo control (una vez finalice el estudio) podrán beneficiarse del programa de ejercicio físico. Aprenderán a manos del entrenador personal cómo modificar su estilo de vida sedentario y cómo realizar ejercicio de una forma beneficosa y segura, según las recomendaciones de las guías actuales, de una forma totalmente gratuita. Además, mejorarán los componentes del síndrome metabólico y su capacidad física, disminuyendo el riesgo de padecer eventos cardiovasculares, diabetes y otras enfermedades que han sido relacionadas con el síndrome metabólico.

**RIESGOS e INCONVENIENTES**

Los beneficios de la realización de ejercicio físico son mucho mayores que los riesgos. No obstante, las actividades están diseñadas para realizar un aumento progresivo de la carga de trabajo en un intento de mejorar su función y la reacción del sistema cardiovascular a estas actividades no puede predecirse con completa precisión. Algunos cambios que pueden producirse son: alteraciones en la presión o frecuencia cardíaca (rápido, lento o inefectivo), o sensación de mareo. También existe un riesgo mínimo de infarto o paro cardíaco. Las posibilidades de estos acontecimientos son mínimas, comparadas con intervenciones de mayor intensidad. El riesgo de padecer un evento de muerte súbita durante la práctica de ejercicio es ínfimo (1 por cada 1,51 millones de episodios de ejercicio). Además existe el riesgo de lesión musculo esquelética: esguinces, contusiones o heridas y problemas indirectos (como artritis o dolor de espalda). Sin embargo, esta intervención se basa en ejercicios de bajo impacto e intensidad, que causan menos impacto en articulaciones y músculos que actividades de mayor impacto o intensidad.

Todos los pacientes estarán estrictamente vigilados durante las sesiones. Si durante alguna de las sesiones se detecta algún efecto secundario grave, se suspenderá inmediatamente la intervención y se tomarán las medidas necesarias. El centro donde se realiza la intervención cuenta con un seguro para hacer frente a posibles eventualidades acontecidas durante la intervención. Todos los acontecimientos que se manifiesten sobre el estudio, se deben comunicar al investigador principal (teléfono: 972 186 922).
PARCIPACIÓN VOLUNTARIA

Su participación en el estudio es voluntaria. Por lo cual, aunque inicialmente aceptara participar, usted podrá solicitar a los responsables del estudio, en cualquier momento y sin necesidad de especificar el motivo, la baja del estudio, así como la eliminación de toda la información recogida, sin que esto percuta en la relación con su médico o su tratamiento.

Puede comentar la información recibida con su familia, su médico o con quien considere oportuno para sentirse bien aconsejado. El médico responsable del estudio le contestará cualquier pregunta o duda que no haya quedado clara.

COMPENSACIÓN ECONÓMICA

La participación en el estudio no será beneficiaria de ninguna compensación económica. Su participación no le supondrá ningún gasto y le serán reintegrados todos los gastos extraordinarios (como comidas y traslados) si usted lo solicita.

Los investigadores no obtendrán beneficio económico con este estudio. En caso de generarse un desarrollo comercial de los conocimientos obtenidos, los posibles beneficios serán destinados a cubrir gastos científicos.

CONFIDENCIALIDAD

Todos los datos de carácter personal e información recogida o generada en el estudio quedarán protegidos de acuerdo con la legislación vigente sobre protección de datos de carácter personal (Ley Orgánica 15/1999, del 13 de Diciembre, de Protección de Datos de Carácter Personal y el posterior Real Decreto 1720/2007, del 21 de Diciembre.) Nadie, excepto su médico y el personal directamente relacionado con este estudio, podrá conocer su identidad. Esta información estará vinculada a un código, para prevenir que la información se conozca. Este vínculo solo lo conocerá su médico responsable. Únicamente las autoridades sanitarias podrán tener acceso a las secciones relevantes de este estudio, si así lo solicitaran.
ANNEX 7: INFORMED CONSENT FOR EXERCISE TRAINING.
HOJA DE CONSENTIMIENTO INFORMADO

Los péptidos natriuréticos como posible mediador de los efectos positivos del ejercicio físico en pacientes con síndrome metabólico: un ensayo clínico controlado y randomizado

Nombre y apellidos del paciente: ______________________________________________________
Fecha de nacimiento: __/__/____ Número de teléfono: ________________________________
Confirme que:
He leído y entendido la hoja de información que se me ha entregado.
He entendido lo que se me pide en el estudio.
He meditado detenidamente mi decisión de participar en el estudio.
He podido hacer preguntas sobre el estudio.
Se han respondido mis preguntas de forma satisfactoria
He recibido suficiente información sobre el estudio
He hablado con (nombre del investigador/entrenador personal/enfermero)
Entiendo que la participación es voluntaria, y que puedo retirarme del estudio cuando quiera, sin que esto repercuta en los cuidados médicos y sin dar explicaciones
En consecuencia,
Doy mi conformidad para participar en este estudio:
☐ Sí ☐ No
Permito que la información que se obtenga de este estudio sea utilizada en investigaciones futuras relacionadas con este estudio
☐ Sí ☐ No
Permito que la información sea introducida en la base de datos del hospital
☐ Sí ☐ No

Firma del paciente participante: ____________________________
Fecha: __/__/____

Firma del investigador: ____________________________
Fecha: __/__/____