APPENDICES

APPENDIX 1

INFORMATION SHEET

Study entitled: "Metabolic flexibility in patients with early triple-negative breast cancer and the possible effect of a physical exercise intervention"

You are being asked to participate in a study entitled: "Metabolic flexibility in patients with early triple-negative breast cancer and the possible effect of a physical exercise intervention". This research has been approved by the Research Ethics Committee of Getafe University Hospital.

Voluntary participation

The healthcare personnel treating you have proposed that you participate in a study to determine the metabolic flexibility of muscle fibres, i.e., to verify the substrate with the highest preference in muscle fibre and the existence or absence of mitochondrial dysfunction due to cancer treatments and the pathophysiology of the disease. In addition, the aim is to find out whether the proposed physical exercise programmes can modify the aforementioned metabolic flexibility. Your participation is, of course, entirely voluntary and you can decide whether or not to participate without this affecting your medical care in any way.

General information

Some authors suggest that physical exercise has a significant influence on glucose levels, insulin resistance, growth factors, fat oxidation ratio, and lactate, among other factors. Alteration of any of these markers could activate tumour signalling pathways, thus representing a risk factor for tumour proliferation. Therefore, it could be concluded that the modulation of metabolism through physical exercise plays a fundamental role in the development of cancer.

Physical exercise is considered a key tool for improving metabolic flexibility in muscle fibre. In response to increased AMPK and PGC-1α activity with training, increases in mitochondrial content and function have been observed in muscle fibre. This has a positive effect on the ability to oxidise fatty acids in the mitochondria, improving insulin sensitivity. The above would improve the ability of muscle fibre, now with a greater number of mitochondria, to oxidise different substrates, thus improving metabolic flexibility and adapting to different energy requirements with activity.

Purpose of this study

This study has two main objectives:

a) To describe the metabolic function of women diagnosed with triple-negative breast cancer at different stages of early disease.

b) To verify the effects of different training interventions on the metabolic function of women diagnosed with triple-negative breast cancer.

Description of the tests

Your participation in the study will consist of undergoing the following assessment tests, which will be repeated four times throughout the study (after diagnosis, at the end of neoadjuvant cancer treatment, after surgery, and at the end of the physical exercise intervention): a biochemical test if you have not had one done at the hospital; a DEXA scan; an electrical bioimpedance test; three questionnaires, EORTC QLQ-C30, EORTC QLQ-BR45 and FATIGA PERFORM, related to quality of life and fatigue; a resting calorimetry test; two isometric strength tests of the pectoral muscles and the anterior thigh muscles; a cycle ergometer CPET using an incremental methodology; and a CPET using a ramp protocol. The complete assessment includes all of the above tests and will be carried out on the same day over approximately two hours for each patient at the European University of Madrid.

Following the patient's surgery, there will be three experimental groups (E1, E2, E3) and a control group (CG). If randomly selected for the experimental group, they will participate in moderate cardiovascular training two days per week, interval cardiovascular training two days per week, or strength training two days per week at the European University of Madrid.

On the one hand, participants in E1 will join a 12-week cardiovascular training programme on a stationary bike at the W associated with $FAT_{máx}$, which was determined in the incremental exercise test after recovery from surgery, two days a week. There will be two weeks of familiarisation, during which the session will last 60 minutes at the intensity described above. Weeks three and four of the intervention will be marked by an increase in volume in minutes while maintaining the intensity, with a total session time of 65 minutes. This will be followed by 75 minutes of work at the W associated with $FAT_{máx}$ over the next eight weeks. Finally, the session will end with 10 minutes of cool-down with joint mobility exercises and active stretching. There will be numerous exercise bikes and a functional room for cardiovascular training for group E1 at the European University of Madrid.

On the other hand, participants in E2 will carry out a 12-week cardiovascular training programme on a stationary bicycle at MAP, which was determined in the ramp exercise test after recovery from surgery, two days a week. Thus, during the first two weeks of familiarisation, ten 1-minute intervals at MAP will be carried out with a 1-minute rest between intervals at 10% of MAP. Subsequently, the interval time will be increased in the following two weeks (weeks 3 and 4), with six 2-minute intervals at MAP with a 2-minute rest between intervals at 10% of MAP. Finally, four 3-minute intervals at MAP will be reached in the remaining eight weeks, with a 3-minute rest between intervals at 10% of MAP. The session will end with a 10-minute cool-down with joint mobility exercises and active stretching. There will be numerous exercise bikes and a functional room for the cardiovascular training of group E2 at the European University of Madrid.

Participants in E3 will follow a 12-week strength programme with progressive loads, training twice a week. Each strength session will begin with joint mobility and muscle activation exercises. This activation exercises will be consisting of core exercises (shoulder girdle, lumbopelvic girdle, and gluteal activation) and plyometrics. There will be a two-week familiarisation period in which the patient's initial level will be taken into account, starting with moderate loads around 6 on the RPE and a high number of repetitions (12) with a high repetitions in reserve (RIR) (4). Between weeks three and four, patients should perform 10 repetitions per strength exercise with a moderate RIR (3) and an RPE of 7. The following eight weeks will progress to high loads with an RPE between 8-9 and a lower number of repetitions (8-6) with a low RIR (2-1), thus attempting

to maximise adaptations to training. They will start with 3 sets during the first six weeks, reaching 4 sets in the remaining six weeks of the intervention.

The exercises to be implemented during the strength intervention are as follows: two knee-dominant lower limb exercises, hex bar squats and front lunges; two hip-dominant lower limb exercises, bilateral glute bridge and unilateral hamstring bridge; two upper limb pushing exercises, bench press and unilateral landmine in knight position; and two upper limb pulling exercises, neutral pull-ups with elastic band and unilateral dumbbell row.

Finally, there will be a CG that will not be included in the intervention programmes. However, they will be informed about the general exercise recommendations for cancer patients. Like groups E1, E2 and E3, they will be evaluated at the end of the exercise intervention. In addition, they will subsequently be offered the programme that has obtained the best results in the metabolic flexibility parameters and the rest of the variables analysed.

The incremental exercise test will be carried out by exercise professionals (physical education and sports instructors) and in the presence of a doctor whenever there is a medium or high risk of cardiovascular events, although it is unusual for any type of setback to occur during these tests.

The exercise sessions will be supervised and designed by physical education instructors, meaning registered professionals who have completed a degree in Physical Activity and Sports Sciences.

Collection and use of biological samples

Glucose, lactate, glycosylated haemoglobin, C-reactive protein and lipid profile tests will be carried out by professionals in the field and in an environment that complies with the specific regulations for their performance, at the European University of Madrid. The second drop of blood will be extracted, discarding the first, for each biochemical parameter by capillary puncture of the middle or ring finger of the non-dominant hand, if possible.

The samples of these biochemical parameters will be used exclusively for the indirect determination of metabolic flexibility. The biological samples will be used solely for this project and will be destroyed at the end of the analysis at the time of collection.

Anticipated risks

The discomfort associated with capillary puncture refers to mild pain at the time of puncture, redness at the puncture site, residual blood drop after extraction, small bruising, and/or minimal local inflammation.

Strength training may pose a certain risk of joint, tendon and muscle stress, which increases the risk of injury and overload (Chen et al., 2021; Kambič et al., 2024). Similarly, training with heavy weights could raise blood pressure and increase the risk of acute cardiovascular events (Chen et al., 2021; Kambič et al., 2024).

There may be a high risk of acute cardiovascular events when exercising if the person is sedentary and has known or hidden heart disease during high-intensity exercise (Riebe et al., 2015).

Strenuous, intense, prolonged physical exercise or exercise with a high demand for eccentric contractions could lead to delayed onset muscle soreness (DOMS) and muscle fatigue, causing a temporary loss in maximum voluntary contraction strength (Dupuy et al., 2018).

Under conditions of prolonged fasting or a high contribution of glucose to energy generation during exercise, there is a certain risk of hypoglycaemia (Davis et al., 2014). However, this risk occurs mainly in diabetic patients (Davis et al., 2014).

Performing a CPET can cause certain changes such as abnormal blood pressure, fainting, or irregular, slowed, or accelerated heart rate (Thompson et al., 2013). There is a minimal risk of sudden cardiac death, specifically 0 to 5 per 100,000 tests, or 0.005% (Thompson et al., 2013; Myers et al., 2009). Similarly, the risk of complications requiring hospitalisation (including severe arrhythmias), acute myocardial infarction, or sudden cardiac death during or immediately after the test is less than 0.2% in the first case and 0.04% and 0.01%, respectively (Myers et al., 2009).

Patients with absolute contraindications for CPET, such as heart failure, myocarditis, acute pericarditis, severe aortic stenosis, aortic dissection, vascular toxicity, severe uncontrolled hypertension, severe uncontrolled cardiac arrhythmias, pulmonary thromboembolism, or severe anaemia, may not be included in the study (Fletcher et al., 2001).

Similarly, patients with relative contraindications for CPET, such as bradyarrhythmias or tachyarrhythmias, moderate valvular stenosis, inability to perform physical or mental exertion, chronic infectious diseases, musculoskeletal disabilities , ventricular aneurysm, second- or third-degree atrioventricular block, or severe hypertension (Fletcher et al., 2001).

Finally, there are some absolute contraindications for strength training. These include uncontrolled hypertension, unstable cardiovascular disease, and acute musculoskeletal injuries. Relative contraindications for this type of training include chronic kidney disease, moderate hypertension, diabetes mellitus, and proliferative diabetic retinopathy (Thompson et al., 2013; Williams et al., 2007; Paulo et al., 2020).

Duration of the study

The duration of the longitudinal study will be a maximum of 2 years, including the descriptive phase and the physical exercise intervention phase, depending on the protocol followed by each patient in their respective hospital in relation to the cancer treatment plan, planned surgery, etc.

The cardiovascular exercise interventions and strength training intervention will have a total duration of 12 weeks, with a training frequency of 2 days per week and a recovery period of at least 48 hours between sessions.

Confidentiality and Data Protection

The Data Controller (Principal Investigator, Dr Lidia B. Alejo), in compliance with Organic Law 3/2018 of 5 December on the Protection of Personal Data and the guarantee of digital rights and Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, undertakes to accept its confidentiality responsibilities.

The data will be included in a file owned by UNIVERSIDAD EUROPEA DE MADRID, S.L.U, with registered office at C/ Tajo s/n, Villaviciosa de Odón, Madrid, which is the recipient of the information provided, for the purpose of being managed by the OTRI – Office for the Transfer of Research Results of the European University of Madrid, for the call for external research projects.

The information obtained will be stored in a database, in computer format, and the data will be processed statistically in a coded form. At any time, the donor shall have the right to access, rectify or cancel the data stored in the database, provided that they expressly request it. To do so, they must contact the principal investigator. The data will be kept under the responsibility of the Principal Investigator of the Study, Dr Lidia B. Alejo.

Insurance

In accordance with current legislation, you will be insured under a policy taken out by the study sponsor to cover any potential risks that may arise from your participation in the study.

At the start of the study, accident insurance will be taken out for the participants. In addition, all members of the research team are registered with their respective professional associations, which means that they all have civil liability insurance covering their professional activities. This insurance is associated with their professional work and covers the institution responsible for the project.

On the one hand, in the event of an accident, immediate attention must be given, the accident must be recorded and reported in the policy's group register, Mapfre must be notified of the accident as soon as possible, and the patient's details and documentation relating to the accident must be provided. This policy covers all types of accidents that occur during the insured person's professional activity within the insured group. In turn, accident insurance covers accidental death (€12,000) and permanent disability according to a scale of up to €12,000. The maximum limit per claim for all insured persons is €1,500,000.

On the other hand, civil liability insurance covers everything that may be claimed from the insured as a result of damages caused unintentionally to clients and third parties due to events arising from their activity, such as: Metabolic flexibility in patients with early triple-negative breast cancer and the possible effect of a physical exercise intervention.

Costs

Participating in this project will not incur any costs.

You will be provided with a copy of this information sheet and your informed consent form to keep.

The researcher providing this information is:

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RESEARCHER TELEPHONE NUMBER

INFORMED CONSENT

I, Mr/Mrs/Ms……
ID/Passport No……Date of birth……..
Address (Street/Road)……..
Town/City……Postcode……..
Country……Email address……

I declare that:

1. I have understood the information contained in the information sheet for the research project "Metabolic flexibility in patients with early triple-negative breast cancer and the possible effect of a physical exercise intervention" that has been given to me, and I have had the opportunity to resolve any questions I may have had about participating in this study.
2. The personal data collected in the study will be used solely for the purposes of the aforementioned project.
3. I may withdraw from the study at any time:
   - Whenever I wish.
   - Without having to provide any explanation.
   - Without this having any negative repercussions.

We inform you that your personal data will be incorporated into a file for which Dr Lidia Brea Alejo is responsible, in order to provide you with the service that is the subject of this communication. If you wish to exercise your rights of access, rectification, cancellation or opposition, you may contact her by email at lidia.brea@universidadeuropea.es

We inform you that you may access, rectify, oppose and/or cancel the personal data in our databases at any time. To do so, you must contact the person responsible for the file and send us a signed request to the address given in the previous paragraph.

We also inform you that recordings or photographs are occasionally taken and used as evidence to justify the project, in which you may appear as a participant. By signing this document, you give your express consent to this.

<u>Data protection:</u>

☐ I consent to the processing of my personal data in accordance with the information provided.

<u>Image rights authorisation:</u>

☐ I consent to the taking, collection and processing of images and/or recordings in accordance with the information provided.

☐ I do not consent to the taking, collection and processing of images and/or recordings in accordance with the information provided.

I wish to give my consent to participate in the study entitled “Metabolic flexibility in patients with early triple-negative breast cancer and the possible effect of a physical exercise intervention” and, to this end, I sign this informed consent document in duplicate.

SIGNATURE OF THE PARTICIPANT/LEGAL REPRESENTATIVE At ……..on the……day of……..in 202…

Signature: …….

I have discussed this project with the patient in understandable language. I believe that I have informed the patient of the nature of the study and that the patient has understood this explanation. I have provided a copy of the information sheet.

SIGNATURE OF THE PERSON RESPONSIBLE FOR THE STUDY At ……..on the……day of……..in 202…

Signature: ……